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REVIEW

Polymeric Scaffolds for Pancreatic Tissue Engineering: A Review

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■ Abstract

In recent years, there has been an alarming increase in the incidence of diabetes, with one in every eleven individuals worldwide suffering from this debilitating disease. As the available treatment options fail to reduce disease progression, novel avenues such as the bioartificial pancreas are being given serious consideration. In the past decade, the research focus has shifted towards the field of tissue engineering, which helps to design biological substitutes for repair and replacement of non-functional or damaged organs. Scaffolds constitute an integral part of tissue engineering; they have been shown to mimic the native extracellular matrix, thereby supporting cell viability and proliferation. This review offers a novel compilation of the recent advances in polymeric scaffolds, which are used for pancreatic tissue engineering. Furthermore, in this article, the design strategies for bioartificial pancreatic constructs and their future applications in cell-based therapy are discussed.

Keywords: diabetes · transplant · scaffold · polymer · pancreas · islets · tissue engineering

1. Introduction

With changing economic development patterns, the world has experienced a steep increase in the number of patients with lifestyle diseases. Diseases associated with lifestyle imbalance include diabetes, hypertension, cardiovascular diseases, and certain types of cancers. Such diseases are associated with a lack of physical activity, unfavorable occupational habits, and increased obesity. Diet and lifestyle play an important role in maintaining physical and mental health [1]. For centuries, infectious diseases have been considered as the main killer around the world. But with non-communicable diseases (NCDs) taking the front seat, it is estimated that by the year 2020 NCDs will cause seven out of ten deaths in developing nations [2]. Diabetes, one of the four priority NCDs, is currently the eighth leading cause of death in both sexes [3, 4]. Initially labeled as a disease of rich countries, diabetes has shown a tremendous increase in the past few years, even in middle income nations. According to the WHO's global diabetes report 2016, a total of 422 million people across the world are currently suffering from diabetes [5]. Its global prevalence increased from 4.7% in 1980 to 8.5% in 2014 (**Figure 1**) [5]. As per the International Diabetes Federation (IDF) report (2015), China ranks first in relation to the number of diabetic patients (between the age of 20 and 79 years) followed by India and the USA (**Figure 2**) [6].

Diabetes mellitus is classified as one of the metabolic disorders characterized by a chronic hyperglycemic condition. This state is mainly attributed to defects in insulin secretion or to the action of insulin in cells or both. Most cases of diabetes are one of two types: type 1 diabetes (T1D) and type 2 diabetes (T2D). There are additional types such as gestational diabetes (GD) and maturity onset diabetes of the young (MODY). T1D, also known as insulin-dependent diabetes mellitus

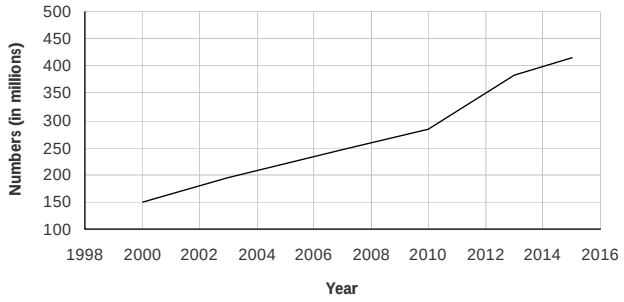

Figure 1. Prevalence of diabetes worldwide. Worldwide increase in the occurrence of diabetes in the past two decades. Source: IDF Diabetes Atlas; http://www.diabetesatlas.org.

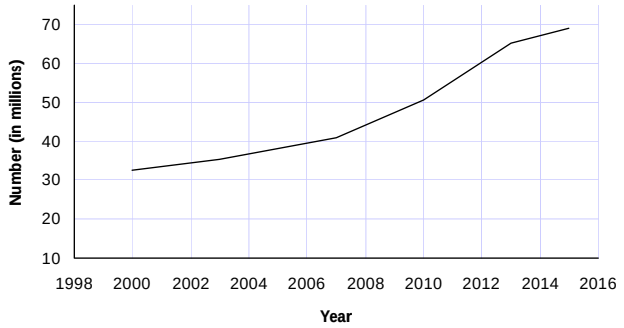

Figure 2. Prevalence of diabetes in India. Increasing similar to worldwide trend. Source: IDF Diabetes Atlas, http://www.diabetesatlas.org.

(IDDM), is an autoimmune condition where the body attacks its own β-cells, destroying them, and rendering them unfit to produce insulin, thereby increasing blood glucose levels [7, 8].

Currently, treatments for diabetes include insulin therapy, drugs such as biguanides, sulfonylureas, megalitinides, thiazolidinediones, alfa gluocosidase inhibitors, and others, whole pancreas transplantation, islet transplantation, and bariatric surgery. Even after the award-winning discovery of insulin therapy in 1921, diabetes treatment has not come a long way [9]. Over the years, significant research has focused on the development of substitute routes for insulin administration like nasal, rectal, and oral [10]. Several insulin release devices such as insulin pumps, pen injectors, and inhalation patches have been engineered to enhance patient amenability [11]. For controlled and tunable release of insulin, carriers made of hydrogels, microspheres, and nanoparticles have been formulated [12].

Although multiple modifications in the source, structure, and delivery mode of insulin have been made to improve the management of diabetics, millions of patients still continue to inject themselves with insulin several times a day. Apart from numerous injections, prolonged usage of insulin can cause diabetic retinopathy, ketoacidosis, weight gain, etc. All these treatment options have serious disadvantages which have led to a search for better options. In recent years, tissue engineering has shown promise in the treatment of various conditions.

Abbreviations:

ANCOVA	analysis of covariance
2D	two-dimensional
3D	three-dimensional
AG-CHNP	agarose chitosan-based nanocomposite
BM-MSC	bone marrow-derived mesenchymal stem cell
ECM	extracellular matrix
EC	endothelial cell
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EGF	epidermal growth factor
FDA	US Food and Drug Administration
FGF	fibroblast growth factor
GD	gestational diabetes
GLP-1	glucagon-like factor 1
HEK	human embryonic kidney
HeLa	cervical cancer cell line (Henrietta Lacks)
IDDM	insulin-dependent diabetes mellitus
IDE1	inducer of definitive endoderm 1
IDF	International Diabetes Federation
IKVAV	isoleucine-lysine-valine-alanine-valine
INS-1	insulinoma cell line
IPN	interpenetrating polymer network
iPSC	induced pluripotent stem cell
Mia PaCa-2	human pancreatic carcinoma cell line
MODY	maturity onset diabetes of the young
MSC	mesenchymal stem cell
NCD	non-communicable diseases
NHS	N-hydroxysuccinimide
NKX2.2	NK2 homeobox protein 2
PAA	polyacrylic acid
PCL	polycaprolactone
PDMS	polydimethylsiloxane
PDX1	pancreatic and duodenal homeobox 1
PEG	polyethylene glycol
PGA	polyglycolic acid
PLA	polylactic acid
PLG	polylactide-co-glycolide
PLGA	polylactic-co-glycolic acid
PU	polyurethane
PVP	polyvinylpyrrolidone
RGD	arginylglycylaspartic acid
RIN-5	rat insulinoma cell
ROS	reactive oxygen species
SC β-cell	stem cell-derived β-cell
STZ	streptozotocin
T1D	type 1 diabetes
T2D	type 2 diabetes
VEGF	vascular endothelial growth factor
WHO	World Health Organization
YIGSR	tyrosine-isoleucine-glycine-serine-arginine

2. Introduction of tissue engineering

Tissue engineering comprises several disciplinary fields including engineering, material science, and life sciences; it aims to produce biologically viable substitutes for tissue and organ regeneration [13]. Tissue engineering provides a means to synthesize substitutes for repair or replacement of tissues or organs damaged due to pathology, trauma, or injury. It provides an alternative to bridge the ever-growing gap between demand and supply of organs for transplantation [14].

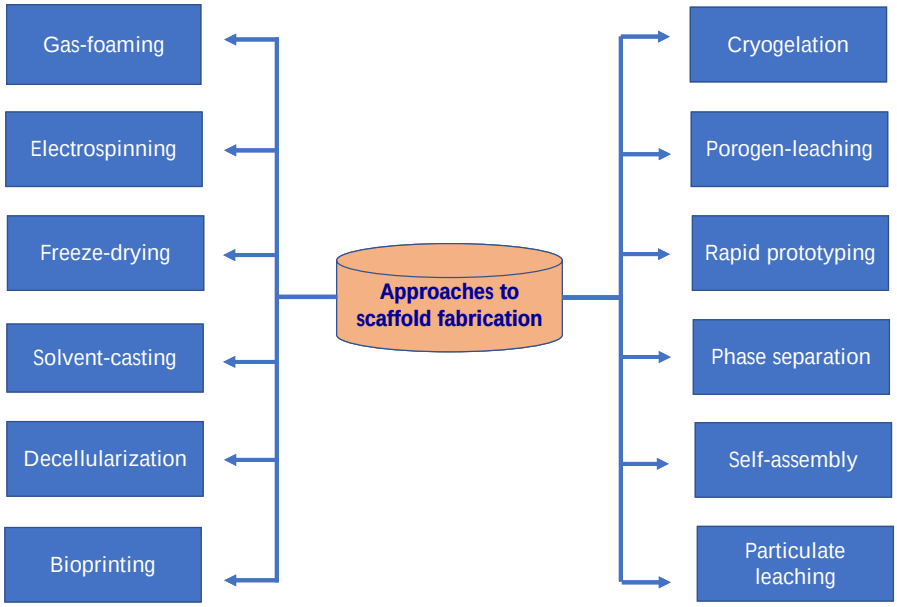

Figure 3. Fabrication techniques for scaffold preparation. Over the last two decades, various approaches have been implemented in the fabrication of scaffolds for tissue engineering, including electro-spinning, phase-separation, solvent casting, freeze-drying, and self-assembly. The selection of the appropriate scaffolding approach depends on the scaffold requirements and tissue-specific considerations.

Major research efforts have focused on an *in situ* tissue engineering approach, with the aim of leveraging the innate regenerative potential of the human body to enable tissue regeneration at the site of injury using bioactive molecule-based cues [15]. The tissue engineering triad comprises a combination of cells, scaffolds, and biologically active molecules or growth factors [16]. Scaffolds are three-dimensional constructs with the prime function of being able to mimic the physico-chemical properties of the natural extracellular matrix (ECM) [16]. For successful application in the field of tissue engineering, scaffolds need to be able to provide structural and mechanical support to the cells and to promote regeneration by effectual delivery of therapeutic molecules [17].

Polymeric scaffolds have been used widely for tissue engineering applications. Biodegradable polymers provide the advantages of enhanced inflammatory tolerance, high biocompatibility, and nontoxic enzymatic degradation *in vivo* [16]. Numerous approaches have been implemented for the fabrication of these biomimetic scaffolds, including electro-spinning, phase-separation, solvent casting, freeze-drying, and self-assembly (**Figure 3**) [18]. Electro-spun nanofibers from biomaterials form structures analogous to the fibrous native ECM, and possess beneficial mechanical properties and enhanced cellular infiltration [19, 20]. Pre-vascularized tissue constructs possessing enhanced cell proliferation have been developed by pre-seeding endothelial cells and fibroblasts on hydrogels [13].

An alternative is provided by scaffold-less tissue engineering. It has been developed by virtue of 3D bioprinting using self-assembling multicellular units as bioink particles, and has been used to realize self-organizing vascular constructs [21, 22]. The latest strategy of 4D bioprinting, which involves time as the additional dimension, has enabled the development of smart biomaterials which can evolve their shapes as a function of time in response to exogenous cues like pH and temperature [23]. Organ decellularization is another recent avenue where organs, decellularized by detergents, retain ECM and vascularization, and hence can be transplanted after *in vitro* recellularization [24]. Hydrogels made from natural and synthetic polymers have been used for encapsulation and to protect the transplanted cells from the host immune system. The permeability of the encapsulating matrix is fine-tuned to block the passage of antibodies and T cells, and at the same

Table 1. Advantages and disadvantages of natural polymers for tissue engineering applications

Polymer	Advantages	Disadvantage
Collagen	Biocompatible and biodegradable [37]	Low mechanical strength [43]
Gelatin	Biocompatible [45] Biodegradable [46] Non-antigenic and non-immunogenic [47-48]	Low mechanical strength [49]
Fibrin	Self-assembly [53] Soft elasticity [53] Low toxicity to cells [54] Good attachment, proliferation and migration properties [54, 55]	Immunogenicity [53]
Agarose	Biodegradability [61] Soft tissue-like mechanical properties [60] Rapid gelling capacity [60]	Low cell attachment and proliferation [14]
Alginate	Non-toxic approach to encapsulate cells [70] Excellent gelling properties [70]	Limited cell adhesion [70]
Silk	Excellent mechanical strength [83] Good biocompatibility [83] Water-based processing [83-84] Simplicity of chemical modification [83] Biodegradability [83]	Stimulates host immune response [92]

time, to allow the inflow and outflow of bioactive signaling molecules, thus aiming to avoid the usage and eventual side-effects of immunosuppressive agents [25, 26].

3. Pancreatic tissue engineering

A surgical cure for diabetes has been proposed by pancreatic transplantation, which is accompanied by long-term immunosuppressive therapy [27]. To reduce the extent of surgical intervention and the risk involved in pancreatic transplantation, new strategies have been developed for islet transplantation [28]. It has been found that immunoisolation of islets, using tissue engineering techniques like encapsulation and coating with semi-permeable and biocompatible biomaterial membranes, minimizes the need for long-term immunosuppression [29].

Cell-based (HEK293) microencapsulation of islets has also been tested which showed sustained release of insulin [30]. Although this technique requires further improvement, islet surface modifications with growth factors such as vascular endothelial growth factor (VEGF) and peptides such as arginylglycylaspartic acid (RGD), isoleucine-lysine-valine-alanine-valine (IKVAV), and tyrosine-isoleucine-glycine-serine-arginine (YIGSR) have already been shown to enhance islet engraftment and reduce immunogenicity in pancreatic islet transplantation [11].

To increase donor tissue sources, transplantation of β-cells derived from stem cells differentiated *in vitro* has become a new focus in diabetes research [31]. Hydrogels and microspheres made of polymers like alginate, polyethylene glycol (PEG), agarose, and chitosan-gelatin among others have been used for β-cell encapsulation. These encapsulated β-cells have been shown to have enhanced viability, cell survival, and insulin-secretory potential. Major efforts have been directed to mimic the islet niche and native interactions in the capsules to improve the efficacy of islet transplantation [11]. Natural and synthetic polymers have been widely vetted as a means of transplantation to enhance the efficacy of islet survival [32].

4. Polymers

Polymers can be categorized as natural or synthetic polymers depending on their origin. Naturally occurring polymers like polysaccharides (chitosan, alginate, hyaluronic acid), inorganic polymers (hydroxyapatite), and natural proteins (collagen, fibrin, silk) exhibit several benefits such as low toxicity, biocompatibility, and enzymatic degradation [16, 33]. Natural polymers also contain bioactive motifs, which help to establish cell-scaffold interactions, thus enhancing tissue functionality [34]. The downsides associated with natural polymers include temperature sensitivity, immunogenicity, and source-dependent heterogeneity (**Table 1**) [17].

The second family of polymers, synthetic polymers, includes alpha-hydroxy acids such as polylactic acid (PLA), polyglycolic acid (PGA), polylac-

Table 2. Advantages and disadvantages of synthetic polymers for tissue engineering applications

Polymer	Advantages	Disadvantages
PGA	Biocompatible [94] Tunable degradation rate [33] Stable three-dimensional structures [33]	Increased release of acidic degradation products [33] Inflammatory response [33] Rapid *in vivo* absorption [33]
PLA	Biocompatible and biodegradable [101] Long half-life [101] Tailorable physico-chemical properties [103]	Hydrophobic nature with low biomimetic and cell adhesion properties [102]
PLGA	High biocompatibility [108] Non-toxic biodegradation [108] Tunable mechanical strength [109] Biodegradation rate [109]	Poor protein absorbance, cell affinity, and surface characters like hydrophilicity [110]
PCL	Biodegradable [16] Low melting point [118] Remarkable blend compatibility, versatile mechanical properties, and viscoelastic properties [118]	Hydrophobic nature, limited bio-regulatory activity, and susceptible to bacteria-mediated degradation [33]
PDMS	High biocompatibility [125] Excellent oxygen solubility [125] Ideal for slow release of compounds [126]	Hydrophobic surface, low cell adhesion [126]
PEG	Low immunogenicity, tissue-like elasticity, well-defined chemistry [130]	Biologically inert, does not support cell growth [129]

Legend: PCL - polycaprolactone, PDMS - polydimethylsiloxane, PEG - polyethylene glycol, PGA - polyglycolic acid, PLA - polylactic acid, PLGA - polylactic-co-glycolic acid.

tic-co-glycolic acid (PLGA) copolymers, and polycaprolactone (PCL) [33, 35]. Synthetic polymers are widely applied in the field of tissue engineering because of their tunable physico-chemical properties. The polyester family of synthetic polymers provides controllable and reproducible material properties, including elasticity and degradability, which are very useful in tailoring matrices with desired functions (**Table 2**) [34]. The lower possibility of infections and risk of immunogenicity give synthetic polymers an edge over natural ones [36].

Using the advantages of both classes of materials, recent work has focused on synthesizing hybrid scaffolds with both natural and synthetic components. Although there have been several prior studies focusing on the selection of the ideal polymer for encapsulation of islets, there has been no review to date which deals comprehensively with the optimal choice of scaffold materials for islets or pancreatic tissue engineering. The present review offers such a comprehensive insight into the application of natural and synthetic polymer-based scaffolds. This review also analyzes critically the problems associated with the construction of the bioartificial pancreas and discusses different design strategies.

5. Natural polymers

5.1 Collagen

Collagen is a structural basement membrane protein and a widely used biomaterial for cell adhesion and proliferation [37]. Being a part of the extracellular matrix, collagen has found wide application in tissue engineering. It has been used for engineering heart valves [38], lung [39], bone [40], and other organs. Several reports have indicated the use of collagen for pancreatic tissue engineering. Jalili *et al*. incorporated fibroblast in type-1 collagen gels. Before solidification, islets were also embedded in the collagen gel. Collagen provides the ECM for islet growth, and fibroblasts maintain matrix integrity. This scaffold showed improved cell survival and insulin secretion. Importantly, incorporation of fibroblasts reduced the number of islets required to reverse diabetes through transplantation [41]. In another study, basement membrane proteins (laminin and heparin sulfate proteoglycan) were combined along with collagen to form gels in which islets were embedded. These cells showed better proliferation, attributed to the reduced caspase-3 expression, and improved cell survival [42].

To carry neonatal porcine islets Ellise *et al*. used scaffolds containing the following constituents:

- Rat tail collagen cross linked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) N-hydroxysuccinimide (NHS)
- A combination of chondroitin-6-sulfate, chitosan, and mouse laminin

The islets survived up to 28 days indicated by positive insulin and glucagon staining. Also, the matrix did not show any signs of inflammation, and the scaffold could maintain its shape and size for over 28 days [43]. Another study illustrated early restoration of euglycemia post-transplantation (from 17 to 3 days) relative to controls using PLG scaffolds coated with collagen-IV, laminin, and fibronectin [44]. The collagen IV-modified scaffolds showed improved islet survival, enhanced islet metabolism, and better glucose-induced insulin secretion.

Collagen alone does not provide the mechanical strength required for pancreatic tissue architecture. Hence, a combination of other polymers such as chitosan, chondroitin-6-sulfate, and laminin or crosslinking has been used to improve the scaffold [43]. Almost all the studies mentioned above showed that incorporating collagen with other basement membrane proteins tended to improve islet survival and function.

5.2 Gelatin

Gelatin, a natural product generated from hydrolysis of collagen, has offered great potential as scaffolding material [45]. Being a natural polymer and having the beneficial properties of biocompatibility, biodegradability, and lack of antigenicity and immunogenicity, gelatin-based scaffolds have shown promising results for tissue engineering of cartilage [46], bone [47], skin [48], and other tissues. Various research groups have shown effective application of gelatin and its blends for engineering islets. Collagen is a component of the basement membrane of ECM in the adult human pancreas, thereby providing gelatin with an advantage over other polymers. One of the major properties required for pancreatic tissue engineering is good mechanical strength. Gelatin alone does not fulfill this criterion. Therefore, various blends of gelatin with other polymers have been used.

Gelatin has been used for encapsulating rat pancreatic islets grown on polyglycolic acid scaffolds. These engineered islets were transplanted into streptozotocin-induced (STZ-induced) diabetic nude mice. The diabetic mice maintained normal glycemia until 120 days of transplantation, with the islets showing potential to secrete exogenous insulin [49]. Muthyala *et al*. used gelatin to synthesize 3D porous interpenetrating polymer network (IPN) scaffolds along with polyvinylpyrrolidone (PVP) using the cross-linkers glutaraldehyde and EDC hydrochloride by freeze-drying [50]. IPN scaffolds displayed ideal properties for tissue engineering with good mechanical strength. Out of the many scaffolds synthesized, one of them (gelatin-PVP-semi-IPN) showed good growth of viable β-cells even up to 30 days [50]. Moreover, the authors showed that a combination approach, consisting of mouse islets grown on the gelatin-PVP semi-IPN scaffold encapsulated in a PU-PVP semi-IPN microcapsule, (a capsule made up of polyurethane extrusion grade Tecoflex 60D (TFPU) and PVP coated with semi-IPN solution), reversed diabetes in rat models for up to 90 days [51].

Previous studies have used gelatin in combination with dextran to produce three scaffolds (DEXGEL). Sodium meta-periodate was used to incorporate the aldehyde group in dextran which could link with the amine group of gelatin, thereby negating the use of additional cross-linkers. DEXGEL served as a platform for differentiation of adipose stem cells into islet-like clusters. These islets provided higher levels of insulin secretion than 2D culture systems [52].

5.3 Fibrin

Fibrin is a protein involved in blood clotting. It has been used widely for tissue engineering applications because of properties such as self-assembly and soft elasticity [53]. Fibrin hydrogel has shown various impressive properties such as low toxicity to cells, good cell anchorage, proliferation, and migration [54, 55]. Fibrin gels have been used to differentiate chemically human endometrial stem cells into pancreatic β-cells using activin A, nicotinamide, fibroblast growth factor (FGF) and epidermal growth factor (EGF) [56]. Insulin secretion was found to be higher in 3D fibrin gels enclosed with differentiated cells than in their 2D counterparts. Khorsandi *et al*. have shown differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs) into insulin-producing cells using 3D culture and fibrin glue [57]. Previously, long-term proliferation of a rat insulinoma cell line (INS-1) on fibrin gel had shown increased insulin secretion in response to glucose stimulation [58].

Fibrin has also been shown to significantly improve insulin secretion in diabetic mice which were transplanted with fibrin-cultured islets. These mice had shown highly vascularized islets along with improved viability [59]. This shows the importance of fibrin in maintaining islet cell viability and angiogenesis. Although the use of fibrin for islet proliferation has brought about much improvement, one of the major drawbacks associated with it is the risk of immune response *in vivo* [53]. Furthermore, the potential application and risks associated with fibrin for islets proliferation and transplantation are not yet fully elucidated.

5.4 Agarose

Agarose, a naturally occurring polysaccharide, is one of the most widely used polymers in the field of tissue engineering. Its favorable properties include biodegradability, soft tissue-like mechanical abilities, and strong and rapid gelling capacity, and make it an ideal candidate for soft tissue engineering [60]. Agarose gel has been used as gene delivery vehicle [60], scaffold for implantation surgery [61], cartilage tissue engineering [62], liver tissue engineering [63], and other applications.

Recently, our group formulated an agarose chitosan-based nanocomposite (AG-CHNP) using a freeze-drying technique. Our scaffold showed good biocompatibility with various cell lines, including HEK, Mia PaCa-2, and HeLa, hemocompatibility, and antibacterial activity. These scaffolds showed continuous increased growth of HeLa for a period of 16 days [14]. We further used AG-CHNP scaffolds for chemical differentiation of BM-MSCs into insulin-producing cells. The differentiated cells showed positive results for the pancreatic markers PDX1 and NKX2.2. The differentiated cells secreted insulin confirmed by western blot (unpublished data). These preliminary results suggest that such agarose-based scaffolds are suitable for pancreatic tissue engineering.

For islet engineering, agarose-agarose islet macrobeads were used to encapsulate porcine islets. These macrobeads were xenotransplanted into pancreatectomized dogs. This, along with anti-inflammatory pravastatin therapy, showed prolonged functionality and biocompatibility of the islets [64]. Luan *et al*. transplanted islets into a prevascularized subcutaneous space. Induction of blood vessels was performed using freeze-dried agarose rods comprising basic FGF (bFGF) and heparin. 1500 islets were transplanted into the prevascularized subcutaneous tissue without using any immunosuppressive regimen. This therapy reverted hyperglycemia, and showed long-term allogeneic islet graft survival and function [65].

One report used agarose microwells made up of polydimethylsiloxane (PDMS) molds for the formation of primary islet aggregates, which are pseudoislets with pre-defined proportions [66]. Dissociated islets, when aggregated in a controlled environment, led to a change in the core mantle arrangement of α- and β-cells, which underwent modification after implantation under the kidney capsule. After transplantation, these islets behaved almost like native islets. This observation demonstrated the importance of cell-to-matrix interaction, and the necessity for the islets to have the appropriate size and shape for the maintenance of their structure and function *in vivo* [66].

Apart from this finding, Ichihara *et al*. used size-controlled pseudoislets from rat pancreas on agarose gel-based microwells. The micromolds were synthesized using soft lithography of different diameters (100, 300, 500 μm). These small islet aggregates showed better insulin secretion and cell survival than medium-sized and large aggregates. Also, native tissue-like cell organization was observed in both small- and medium-sized islet aggregates [67]. This study highlighted the role of size in islet transplantation. Recently, a novel approach of combining agarose gel scaffolding with BM-MSCs showed improved insulin secretion compared to controls where islets were grown on agarose gel only. This study highlighted the role of BM-MSCs and agarose gel in improving the overall functionality of islets. It is suggested that BM-MSCs provide growth factors and paracrine signaling, and agarose gel allows cells to absorb nutrients in an unlimited manner which improves islet function [68].

5.5 Alginate

As mentioned above, microencapsulation is a process of entrapping cells or tissues within a polymeric membrane that acts as immunosuppressive barrier [69]. Currently, much research has been done in microencapsulating β-cell grafts to allow easy transplantation, immunoprotection, and the use of non-human islets [70]. A commonly used polymer for microencapsulation is alginate, a polysaccharide isolated from brown sea weed [71]. It has gained tremendous popularity after Lim *et al*. used islet-encapsulated alginate beads as artificial pancreas [72]. From then on, alginate has been widely used.

Alginate hydrogels have found application as beads, delayed gelation systems, macroporous scaf-

folds, 3D printed scaffolds, etc. [73]. Although alginate beads enable a rapid and non-toxic encapsulation of cells, their property of limited cell adhesion is a huge disadvantage for their wide application in tissue engineering. Therefore, in the field of islet tissue engineering, alginate has been mainly used for microencapsulation only. A breakthrough in alginate-based encapsulation techniques was made in 2010 when Opara *et al*. suggested a multi-layer model of bioartificial pancreas containing two alginate layers separated by a semi-permeable membrane made up of poly-l ornithine. The inner layer was used to encapsulate the islets, and the outer layer for the adjunct of angiogenic proteins. These microcapsules were implanted into the omental pouch of rats. The authors reported that the use of such alginate beads enabled controlled delivery of growth factors and initiation of blood vessel formation, thereby improving graft viability and function [74].

Gelation of alginate takes place in the presence of ions (Ca^{2+} or Ba^{2+}). But an ionically bound alginate hydrogel of this kind may not be able to withstand the mechanical stress associated with implantation. Therefore, alginate was modified by incorporating a carboxylic group into alginate backbone, and covalent linking to modified PEG (phosphine group at the end) using Staudinger ligation. This hydrogel had better stability and cell attachment than the alginate controls [75]. The microencapsulation system proposed by Opara *et al*. was improved with a thick and cross-linked outer alginate layer. This procedure helped to maintain the stability of the system for a longer period; the microcapsule remained intact even after 90 days of transplantation. This work also suggested the omental pouch as a potential implantation site for islet transplantation [76].

Richardson *et al*. demonstrated a stage-wise directed differentiation of alginate-encapsulated human embryonic stem cells into islet-like cells. Clear viable colonies were evident after differentiation and maturation. Encapsulated cell differentiation resulted in strong maturation marker expression and improved hormone secretion as compared to their 2D counterparts [77]. Additionally, 3D bioplotting has been used to formulate alginate-gelatin porous scaffolds which can be used as extrahepatic islet-delivery systems (bioplotting is a technique that causes extrusion polymers to create custom-engineered scaffolds). When islets were removed from the hydrogel, they showed full functionality [78]. This study is one of the most recent reports on the use of 3D bioplotting for islet engineering.

Another recent study proved the benefits of a modified form of alginate, triazole-thiomorpholine dioxide (TMTD) alginate, for islet implantation [79]. Emphasizing that the size of the microspheres affects the immunological response to the implants, human embryonic stem cell-derived β-cells (SC β-cells) were encapsulated in 1.5 mm TMTD alginate spheres, which showed better glycemic control than the conventionally used 500 µm alginate spheres. This was the first study to report long-term glycemic control in immune-competent mice containing SC β-cells [79]. This report highlights the role of alginate and its derivatives as an immuno-isolatory device in a xenotransplantation setting.

Alginate-encapsulated islets have also been used for clinical applications in patients with type 1 diabetes by various groups. They have shown long-term stability of the capsule *in vivo* with continuous reduction of exogenous insulin [70]. However, a perfect site of implantation, which overcomes all the disadvantages, is yet to be found [69].

5.6 Silk

Silk protein is commonly used in the textile industry; it is produced by silk worms and spiders. The fibrous protein in it native form consists of a component (sericin) which can elicit an inflammatory response [80]. However, this component can be removed by the process of alkali- or enzyme-based “degumming”.

Apart from textiles, silk is also applied in tissue engineering and drug delivery. It offers various outstanding properties which amplify its role as a biomaterial. One of these beneficial properties for tissue engineering is its excellent mechanical strength, which is higher than that of Kevlar, a synthetic fiber used as a reference point in fiber technology [81]. Apart from this advantage, silk has further beneficial properties that simplify handling, including good biocompatibility, water-based processing, chemical modifiability, and biodegradability [81, 82]. Silk can be molded into any form such as films, electro-spun fibers, hydrogels, scaffolds, and particles.

In the field of tissue engineering, silk (alone and in combination with other polymers and nanostructured fibers) has been used for wound healing [83] as well as the regeneration and reconstruction of bones [84], tendons and ligaments [85], urethra [86], cartilage [81, 87], and other tissues. Silk has also been widely used for pancreatic tissue engineering.

Silk hydrogels have been used to encapsulate mice islets. These hydrogels provided a 3D environment in which the islets could maintain their viability and functionality. In the normal pancreas, islets are surrounded by ECM-containing collagen, laminin, and fibronectin which help in cell adhesion and proliferation. To mimic a similar environment, extracellular proteins and secondary stromal cells were incorporated in silk hydrogel which showed enhanced islet function [88]. Do *et al*. showed that oral ingestion of silk fibroin hydrolysates helps in maintaining pancreatic β-cell integrity, and improves insulin secretion by increasing β-cell mass in hyperglycemic mice [89].

Co-encapsulation of β-cells and mesenchymal stem cells (MSCs) using silk hydrogels has also been explored. Though silk is a magnificent biomaterial, it may still stimulate host inflammatory responses which harm islet growth. However, the presence of MSCs reduces this effect because of their immunomodulatory and angiogenic properties. This multi-dimensional approach has proved successful in terms of graft functionality and revascularization, with an undesirable drawback of bone differentiation [90]. Recently, Kumar *et al*. microencapsulated silk scaffold with alginate and agarose. This scaffold showed sustained growth for rat insulinoma cells (RIN-5). Rat β-cells also showed better growth on the 3D scaffold as compared to its 2D counterpart which was confirmed by expression of primary pancreatic genes [91].

6. Synthetic polymers

6.1 Polyglycolic acid

Polyglycolic acid (PGA) is a biocompatible polymer approved by the US Food and Drug Administration (FDA). It is obtained by ring cleavage polymerization of glycolide. PGA hydrolyses *in vivo* to give glycolic acid, which is a metabolite in the citric acid cycle, thus resulting in low toxicity [92, 93]. PGA has a wide range of applications in the field of tissue engineering due to its tunable degradation rate and intrinsic tendency to form stable 3D structures [33]. However, PGA undergoes rapid absorption *in vivo*, causing failure of the scaffold. Also, inflammatory responses are provoked because of increased release of acidic degradation products. Combination of PGA with several copolymers such as PLGA or PEG has been shown to enhance its beneficial physical and mechanical properties [33]. PGA has been widely used to make bioresorbable sutures and cartilage regeneration [16].

A hybrid scaffold of collagen and PGA with basic fibroblast growth factor has been developed to promote wound healing in type 2 diabetic mice. This hybrid matrix has enhanced compression strength, thus suppressing wound contraction, while also inducing angiogenesis and granulation tissue formation [94]. A study by Chun *et al*. showed that the islet cells grown on PGA scaffolds functionalized with a layer of poly-l-lysine enhanced the surface activity and adhesion capacity of PGA scaffold, and promoted cell proliferation. The PGA scaffold was also shown to provide superior nutrient absorption and metabolite excretion to the cultured islets, providing an appropriate microenvironment for their growth and survival. The cultured islets exhibited enhanced viability, improved morphology, and increased glucose-stimulated insulin secretion [93].

The viability of PGA islet grafts transplanted into the leg muscles of rats with STZ-induced diabetes has also been investigated. This scaffold provided a compatible 3D microenvironment with visible adhesive growth of islets on the scaffold and an adequate supply of blood and nutrients. The results showed increased insulin secretion and significantly decreased blood glucose concentration in rats transplanted with PGA islet grafts as compared to controls [95].

Recently Li *et al*. used PGA scaffolds for increasing the efficacy of islet coating by endothelial cells (ECs). Coating islets with ECs has been shown to improve revascularization and to reduce initial inflammatory response. Due to the presence of PGA scaffolds, enhanced coating efficiency of ECs on the islets was observed. Islet functionality was also improved with enhanced glucose-stimulated insulin release. The authors thus recommended the use of PGA scaffolds in pre-transplant culturing of islet cells and ECs [96].

6.2 Polylactic acid

Polylactic acid (PLA) is also approved by the FDA. It is an aliphatic polymer widely applied in the field of biomedical devices and tissue engineering [97]. PLA hydrolyses *in vivo* to release lactic acid, which becomes incorporated into the citric acid cycle and is naturally excreted, thus making PLA biocompatible and biodegradable in nature [98]. However, numerous surface treatments need to be implemented to hydrophobic PLA to impart enhanced biomimetic and cell adhesion properties [99]. PLA has tunable and versatile physical and chemical properties, and can be molded to take on a myriad of shapes, including microspheres, scaf-

folds, sutures, and nanoparticles [100]. Taking advantage of its long half-life, PLA has been extensively used in fabrication of long-term implantable devices for therapeutic applications [98]. PLA and its copolymers are extensively applied in tissue engineering, including skin grafting and the regeneration and reconstruction of bone, spinal cord and nerve tissue [100].

In the field of pancreatic tissue engineering, the potential therapeutic application of PLA microspheres has been studied in the treatment of diabetic periodontitis. The microspheres, loaded with 25-hydroxyvitamin D3, were shown to prevent inflammatory responses and bone loss in rats with diabetic periodontitis [101].

PLA-PEG-based nanoparticles have also been used as a means for subcutaneous delivery of insulin. Nanoparticles, loaded with 50 IU of insulin per kg, were shown to control blood glucose levels, thereby restoring normoglycemia in diabetic rats. These biodegradable nanoparticles proved to be non-toxic in nature; they are thus qualified as potential candidates for parenteral insulin therapy [102].

Kasujo *et al*. described the application of PLA-based porous capsules to obtain a vascularized microenvironment for extrahepatic islet transplantation. The bioartificial cavity showed numerous vessels and guided infiltration of the host's connective tissue cells and vascular endothelial cells with no significant infiltration by inflammatory cells, providing a favorable microenvironment for islet transplantation [103]. A 3D delivery system has been developed which can be used for encapsulation and implantation of pancreatic cells. The PLA-based nanogland provided support to islet-like aggregates derived from differentiation of human MSCs, enhancing their viability and maintaining their function *in vitro*. The nanogland provided steady secretion of insulin, demonstrating potential benefits for diabetic cell therapy [98]. Recently, a 3D printed encapsulation system has been formulated using polylactic acid for subcutaneous implantation of pancreatic islets. After surface treatment was employed to functionalize the system, it was implanted with VEGF-enriched platelet gel to enhance vascularization. This system enabled transcutaneous refillability and potential retrievability of the graft [99].

6.3 Polylactic-co-glycolic acid

Polylactic-co-glycolic acid (PLGA) is an FDA approved copolymer obtained by ring-opening copolymerization of lactide and glycolide [104]. PLGA has been widely used in varied forms such as films, porous scaffolds, hydrogels, and microspheres for biomedical tissue engineering and drug delivery purposes due to its high biocompatibility and non-toxic biodegradation [36]. An additional advantage of the physico-chemical properties of PLGA is the tunable mechanical strength and biodegradation rate achievable by altering the PLA:PGA ratio [35]. However, PLGA has adverse surface characters such as hydrophilicity, protein absorbance, and poor cell affinity [105]. Numerous surface modulation strategies like surface immobilization, physical adsorption of bioactive molecules, plasma treatment, and incorporation of other biocompatible materials into the PLGA matrix have been tested to make the interface between PLGA and its environment more biomimetic which improved cell affinity [36].

Recently, biocompatible PLGA scaffolds have been produced using 3D printing for use in tissue engineering [106]. Electrospun PLGA-based hybrid nano-fibrous membranes and scaffolds have been widely used for skin, bone, nerve, and soft tissue engineering applications [105].

In the field of pancreatic tissue engineering, micro-porous, biodegradable PLGA has been successfully utilized as a platform for islet transplantation in mouse models [107]. Salvay *et al*. explored the effects of PLGA scaffolds with adsorbed ECM components on the survival of transplanted islets. It appeared that adsorption of these proteins by the scaffold enhanced the efficacy of islet grafts and significantly decreased the time needed for the reversal of diabetes in mice [108]. The effects of integrated ECM components on long-term maintenance of human pancreatic islets cultured in a micro-fabricated PLGA scaffolds have also been investigated *in vitro*. The PLGA scaffold provided a viable niche, with the *in-vitro*-cultured islets displaying insulin release profiles characteristic of native islets [109].

Kheradamand *et al*. demonstrated the use of PLGA scaffolds as an extra-hepatic site for islet transplantation [110]. The addition of ethylcarbodiiminde-fixed (ECDI-fixed) donor splenocyte infusions to the PLGA scaffolds enhanced the efficacy of tolerance induction *in vivo*, and indefinite normoglycemia was maintained in diabetic mice models [110]. Bioresorbable PLGA microspheres have been designed for encapsulation and sustained administration of β-cell-proliferative compounds to intact mouse islets in culture [111]. The improved bioavailability of the mitogen to β-cells *in vivo* may lead to increased β-cell proliferation, and may thus be regarded as a therapeutic appli-

cation in the restoration of normoglycemia in diabetic patients [111].

Recently, Liu *et al*. investigated the fabrication of artificial islet tissues using a fibroblast-modified PLGA membrane for differentiating pancreatic stem cells into insulin-producing cells. This construct secreted insulin and was shown to reduce blood glucose levels in diabetic nude mice. The modified PLGA membrane showed higher compatibility, improved proliferation, and increased viability of pancreatic stem cells compared with the unmodified membrane. Also, it had an enhanced histocompatibility with nude mice [112].

6.4 Polycaprolactone

Polycaprolactone (PCL) is a hydrophobic, biodegradable FDA-approved polymer prepared by ring-opening polymerization of ε-caprolactone in the presence of SnO_2 and heat [16]. PCL has gained an edge in the field of biomedical research because of its low melting point, remarkable blend compatibility, and viscoelastic properties. PCL has been widely used in drug delivery systems as surgical sutures and scaffolding material for tissue engineering because of its tunable degradation rates and beneficial mechanical properties [113]. Drawbacks associated with PCL include hydrophobicity, limited bio-regulatory activity, and susceptibility to bacteria-mediated degradation [33]. To enhance favorable cellular responses, various functional groups have been incorporated into the polymer, making it more hydrophilic and biocompatible [113]. PCL and its copolymers such as PCL-PEG and PCL-PLA have various applications in cartilage, bone, and peripheral nerve regeneration [16].

Nano-fibrous PCL scaffolds have been used for differentiation of human induced pluripotent stem cells (iPSCs) into definitive endoderm cells using inducer for definitive endoderm 1 (IDE1). Electrospun PCL scaffolds exhibited more pores, decreased toxicity, and reduced thickness of the nanofibers, enabling more surface space for cellular proliferation and attachment [114]. A composite hydrogel made from polycaprolactone (PCL) and polyacrylic acid (PAA) is applied in oral delivery of the drug gliclazide, which is used in the treatment of type 2 diabetes. The balance of hydrophobic PCL with hydrophilic PAA provided the property of the hydrogel that controls swelling. The PCL/PAA hydrogel offered a controlled release of the drug and was shown to enhance its bioavailability, resulting in reduced glucose levels [115].

PCL is also applied in diabetic wound healing. Gholipour-Kanani *et al*. blended PCL with chitosan to avoid the use of chemical cross-linkers and achieve a nano-fibrous scaffold with sustainable integrity in aqueous media. This poly(caprolactone)-chitosan-poly(vinyl alcohol) (PCL:Cs:PVA) scaffold was found to promote diabetic wound healing because of its biocompatibility and structural similarity to native ECM [116]. Ranjbu-Mohammadi *et al*. showed the application of curcumin-loaded poly(ε-caprolactone) (PCL)/gum tragacanth (GT) (PCL/GT/Cur) nanofibers in the field of wound healing. The antibacterial nanofibrous membranes enhanced the healing process by simulation of native ECM, presence of curcumin and GT, and improved mechanical stability of the scaffolds because of the presence of PCL.

Tissue-engineered scaffolds were also shown to decrease blood glucose levels in rat models [117]. A current finding highlighted the use of heparanized ring-shaped PCL scaffolds functionalized with VEGF for carrying islets in an alginate core. Vascularization was successfully induced throughout the scaffold by the presence of immobilized VEGF. The embedded islets were shown to maintain their viability and functionality, responding normally to glucose stimulations, and at the same time, possessing obvious immune protection properties. The scaffold demonstrated improved revascularization; it may thus be used as potential vessel for subcutaneous islet transplantation [118].

Recently, Smink *et al*. demonstrated the use of a modified PCL, poly (D,L-lactide-co-ε-caprolactone) (PDLLCL) to create a scaffold which acted as an artificial and retrievable subcutaneous transplantation site for pancreatic islets. PDLLCL was shown to be compatible with islet viability and functionality. Also, islets cultured on PDLLCL exhibited comparatively more insulin granules and lower release of immune system-provoking double-stranded DNA, suggesting PDLLCL as a suitable scaffold with potential for application in the treatment of type 1 diabetes [119].

6.5 Polydimethylsiloxane

Polydimethylsiloxane (PDMS), a silicon based organic polymeric compound, has been commonly used as surfactant, stamp resin for soft photolithography, and other applications. Its superior properties make it a better choice for tissue engineering than other synthetic equivalents. PDMS has high biocompatibility, biostability, and oxygen solubility, which makes it a perfect candidate for implantation [120]. PDMS was used to construct a macroporous scaffold via solvent casting and a particulate leaching method. PDMS has a hydro-

phobic surface which is ideal for slow release of compound, but does not support cell adhesion. Therefore, fibronectin was added to the surface of PDMS scaffolds to make it hydrophilic.

Islets loaded onto PDMS scaffolds and implanted into the omental pouch showed good islet retention and long-term normoglycemia. Interestingly, islets on the scaffold showed enhanced viability and function under low oxygen tension compared to 2D controls [121]. Another study group seeded a fibrin platelet-derived growth factor hydrogel loaded with islets onto the PDMS scaffold and transplanted it into mice. This system helped to reduce the time required for attaining normoglycemia, and enabled increased vessel branching [122].

Recently, PDMS scaffolds have been used for delivery of anti-inflammatory agents such as dexamethasone and fingolimod [123, 124]. Dexamethasone was added to PDMS scaffolds in various quantities. Low concentration of dexamethasone showed improved islets engraftment, but higher concentrations were found to be detrimental as they alter glucose-induced insulin secretion by suppressed activation of the PLC/protein kinase C signaling system [123, 124]. Fingolimod exhibited persistent release, but at a very low concentration (0.1% w/w), which does not have any significant effect on the islets [125].

6.6 Polyethylene glycol

Polyethylene glycol (PEG) is one of the most popular synthetic polymers used for tissue engineering applications. PEG is a low-immunogenic material, has tissue-like elasticity, and its chemistry is well-defined, which provides an advantage over other polymers for islets engineering [126]. PEG has been used in the form of scaffolds and encapsulating agents for islet transplantation. As PEG is biologically inert, it does not support any form of cell growth. Therefore, for use as a scaffold, it must be augmented with another co-polymer.

Mason *et al*. used collagen fibrils in PEG hydrogels, and studied their effect on encapsulated embryonic pancreatic precursor cells. The cells on these scaffolds showed high glucose responsiveness, and had an improved level of insulin gene expression [127]. PEG scaffolds have also been supplemented with fibrin ribbons, which were used for co-culturing endothelial cells and islets [128]. Endothelial cells were encapsulated within the fibrin ribbons, and islets were implemented in the PEG hydrogel. The results suggested an optimum growth for both cell types, penetration of endothelial cells into the hydrogel, and improved vascularization.

A major problem associated with islet transplantation is the requirement for large numbers of islets. To overcome this problem, surface modifications of islets are currently being tested. The main objective of this technique is to reduce the number of islets required for transplantation [126]. Glucagon-like peptide 1 (GLP-1) is produced by the L-cells of the distal ileum, and is an insulinotropic ligand. Kizilel *et al*. directly immobilized GLP-1 on the surface of islets by layer-by-layer assembly of biotin-PEG-NHS, streptavidin, and biotin-PEG-GLP-1. Coated islets showed better insulin secretion in response to high glucose than control islets, which proved the efficiency of this technique. This study also addressed the issue of donor shortage as it required lower numbers of transplanted islets to achieve normoglycemia [129]. Another study using PEG as an encapsulating agent developed a device which had rat islets growing on an acellular scaffold and encapsulated in a PEG/VA semi-permeable membrane. This device was implanted in diabetic rats, and showed a reduction in insulin requirement for at least 2 weeks, restoring partial insulin secretion. To achieve complete euglycemia, the question of the optimal islet number to be transplanted needs further investigation [130]. PEG-based hydrogel microwells have also been developed using photolithography. MIN6 β-cells were seeded on the microwells, and maintained for 5 days preceding retrieval and encapsulation. This PEG-based microwell consistently demonstrated successful formation of MIN6 aggregates. Also, the encapsulated MIN6 aggregates showed better insulin secretion and positive expression of the intracellular binding protein E-cadherin as compared to single cell encapsulations [131].

One of the major causes of islet loss after transplantation is hypoxia which affects the longevity of the implant. Therefore, to facilitate short oxygen supply to the islets, PEG-stabilized hemoglobin had been used as an artificial oxygen carrier [132]. But this system does not support long-term use because of continuous conversion of hemoglobin to methemoglobin by autoxidation and free radical damage, which is deleterious to the cells. Therefore, PEG-based hemoglobin conjugates cross-linked with antioxidant enzymes (superoxide dismutase and catalase) have been used [133], and demonstrated excellent protection against free radicals and oxygen-induced stress in RINm5F cell line. The viability of RINm5F cells was higher and the generation of reactive oxygen species (ROS) was reduced for cells treated with the conjugates.

The results also showed sustained or increased insulin release from the treated islets under partial oxygen pressure situations. This study provided insight into the value of PEG-based conjugates for preventing hypoxia-induced graft failure. Another attempt to prevent post-transplantation islet loss was made by Golab *et al*., where islets were immunoprotected by coating them with Treg cells conjugated with biotin-PEG-SVA (succinimidyl valeric acid ester). This approach was found to be favorable compared to that using biotin-PEG-NHS for coating pancreatic cells with Treg cells, and showed slightly improved insulin secretion [134].

PEG hydrogels have also been used for encapsulation of islets in combination with BM-MSCs, GLP-1, and ECM-based cell adhesion ligands [135]. Insulin secretion could be increased 7-fold by the synergistic effects compared with islets alone, islet functionality and viability could be improved, and MSCs have shown immunomodulatory effects. As mentioned previously, angiogenesis plays a major part in maintaining islets functionality. Pancreatic islets comprise only 1-2% of the total pancreatic cell population, but require as much as 15-20% of the total pancreatic blood supply [136]. Therefore, maintaining similar angiogenic effects post-transplantation is absolutely required. Phelps *et al*. created PEG hydrogels with mild maleimide-thiol cross-linking. These scaffolds were further modified by the addition of RGD motif for cell adhesion and VEGF for vascularization [137]. This study highlighted the use of mesentery as a transplantation site which is far less invasive than hepatic portal transplantation. This scaffold and the delivery strategy showed beneficial outcomes in terms of vascular invasion and insulin secretion. This research also negated injection of islets into the blood stream which can cause immediate inflammatory reaction. Recently, a blend of synthetic PDMS and natural PEG polymer was used for transplantation of islets into epididymal fat pad [138]. Islets were mixed with PEG and then included in photo-linked PDMS molds. As the islets were encapsulated in PEG and PDMS, they were protected from immediate inflammatory attack by the immune system. An interesting finding of this study was that glucose tolerance test revealed normoglycemia within 90 minutes of transplantation.

7. Generating a functional pancreatic construct

Diabetes is one of the leading causes of death in the world with an increasing global prevalence. The available treatment options have their own set of serious implications. Therefore, the focus of current research has shifted to the search for new and safe options which include the bioartificial pancreas. Research associated with the development of the artificial pancreas has seen numerous changes over the years. Various approaches include the use of polymer-based scaffolds, organ decellularization, scaffoldless tissue engineering, bioprinting, and encapsulation.

Polymeric tissue engineering involves the synthesis of an artificial scaffold using natural and/or synthetic polymers and a cross-linker. Various scaffolding approaches are available, including freeze drying, electrospinning, solvent casting, and others [18]. Both natural and synthetic polymers have their own set of advantages and disadvantages.

One of the major problems associated with pancreatic tissue engineering is the complexity of the organ itself. The pancreas contains both an exocrine section (including ductal and acinar cells) and an endocrine section (including the islets of Langerhans) [139]. Though β-cells are the major players in glucose metabolism, other small members of the islet cell family also play a critical part in the overall islet functioning [140]. Therefore, multiple cells types need to be considered in the process of islet graft engineering, which further complicates the work.

Another important factor is angiogenesis. Since islets require a much greater blood supply than any other pancreatic cell compartment [137], the choice of transplantation site becomes important to allow the transplanted construct receiving sufficient blood supply for survival. Various sites for transplantation have been examined, including the peritoneal cavity [122], hepatic portal vein [118], subcutaneous space [65], and subcapsular space of the kidney [41, 49]. Some sites such as omental pouch [76] and mesentery [137] have shown promising results, but the problem of hypoxia and shortage of blood supply is yet to be resolved.

Finally, the islets themselves are a cause of concern for pancreatic engineering since they have poor viability and stability *in vitro* [141]. The limited supply of islets intensifies this problem.

8. Conclusions

The construction of a bio-artificial pancreas is subject to a number of difficulties that need to be overcome. These difficulties include:

- Choice of cell type

- Culture environment
- Site of implantation
- Scaffolding approach
- Requirement of encapsulation

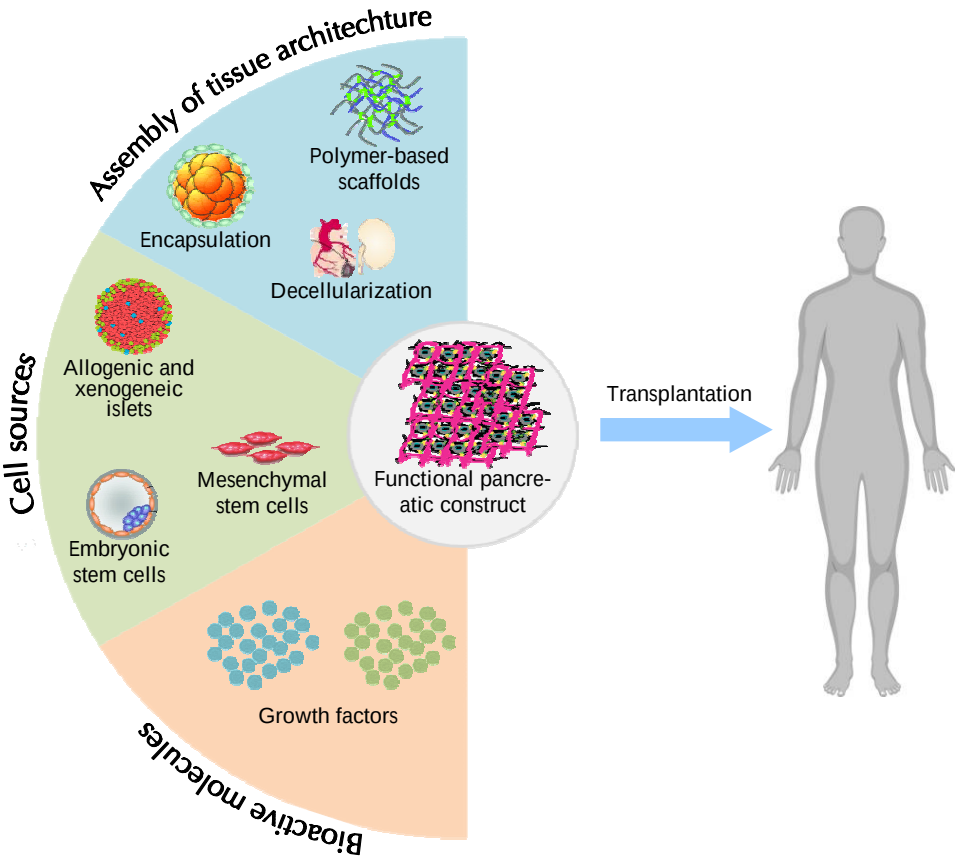

Figure 4. Advances in pancreatic tissue engineering. Various cell sources have been used for pancreatic tissue engineering, including allogeneic and xenogeneic islets (porcine and murine), mesenchymal and embryonic stem cells. Different growth factors have contributed to enhance the stability and proliferation of islet transplantation. Several scaffolding approaches have been employed to mimic the native microenvironment. Micro- and macro-encapsulation of transplanted islets has improved their overall viability and functionality.

Various categories of cell type have been tested for pancreatic tissue engineering and implantation, including allogeneic, xenogeneic, and alternative sources (embryonic stem cells, MSCs) [57, 64, 65, 79]. Although allogeneic and xenogeneic islets have shown promising results, limited availability and poor stability post-isolation have restricted their usability. Therefore, stem cells have received much attention in current research as they have shown promising differentiation potential [31]. While embryonic stem cells are banned in various countries because of the ethical issues, MSCs isolated from different sources have been widely studied [52, 57].

After selection of the cell type, it is important to choose the appropriate culture environment, i.e. whether to culture pancreatic cells individually or co-culture them with other cell types. Transplantation of β-cells alone has shown limited success. Co-culture with other cell types such as fibroblasts or MSCs have shown improved viability, functionality, and insulin secretion [41, 68, 90]. However, the major problem associated with islet transplantation is still substantial cell loss post-isolation and again post-transplantation due to hypoxia-induced apoptosis, loss of suitable microenvironment, and immune response [142-144]. Therefore, current research has been actively focusing on the use of encapsulation that is able to prevent the transplanted cells from direct crossfire from the host's immune system. Moreover, growth factors and angiogenic factors encapsulated within the construct may help to maintain the viability and functionality of the islets [74].

There are many approaches that are under consideration for assembly of a functional bio-artificial pancreas **(Figure 4)**. Considering the organ complexity and factor-dependent stability of the construct, research in the field of pancreatic tissue engineering has come a long way. Advances in tissue engineering and nanotechnology have provided tremendous insight into how we can improve the current approaches for creating a functional bio-artificial pancreas.

We are still awaiting the creation of an appropriate scaffold that can act as a perfect environment for the growth of cells. Various polymers

have been tried and tested for their application in scaffold designs. However, both natural and synthetic polymers fail to address all major requirements of an optimal scaffold for pancreatic tissue engineering. As the scaffold acts as a natural environment for the growth of cells, characteristics such as biocompatibility, biodegradability, vascularization, toxicity, and immunogenicity are critical [16]. One obstacle to finding the perfect polymer for pancreatic tissue engineering is that no single polymer has been studied intensively enough to learn whether it meets all the abovementioned requirements.

As a result of our review, we found silk to be one of the most appropriate polymers for scaffold synthesis. Silk is well studied in the context of pancreatic tissue engineering, and found to be biocompatible and biodegradable [81, 82]. Apart from these advantages, the simplicity of chemical modification and its superior mechanical strength give it a small advantage over other natural polymers [81]. Silk hydrogels have been shown to provide a suitable environment for islets, and enable good islet viability and functionality [88]. Despite these favorable properties, its major drawbacks are its immunogenicity [90] and lack of evidence that silk scaffolds alone can induce angiogenesis. Several attempts have been made to overcome these shortcomings. While reduced immunogenicity and preliminary angiogenesis were observed when MSCs were co-cultured with β-cells, this approach led to unfavorable osteogenesis and chondrogenesis [90]. Additional work may focus on avoiding this colateral differentiation. A recent attempt to suppress immunogenicity was the macroencapsulation of silk scaffolds using alginate and agarose. This study showed positive results with respect to reduced immunogenicity [91]. Future research is necessary to improve the immunomodulatory effect of encapsulated silk scaffolds and to improve the overall viability and functionality of the transplanted islets.

As mentioned above, silk fulfills most of the criteria for application as a scaffold in engineering the bio-artificial pancreas. Since it is well studied and documented, silk may be a forerunner for scaffold design. However, future research on other polymers is also necessary since most have not been studied intensively and their complete capabilities have not been determined. It is thus impossible today to determine the optimal candidate. Future studies aimed at fully characterizing the available polymers and overcoming the existent limitations associated with scaffold fabrication may provide new avenues in the construction of the bio-artificial pancreas to prepare it for routine clinical application.

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■ References

1. **World Health Organization.** Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. 2003.
2. **Boutayeb A.** The double burden of communicable and non-communicable diseases in developing countries. *Trans R Soc Trop Med Hyg* 2006. 100(3):191-199.
3. **UN General Assembly.** Political declaration of the high-level meeting of the general assembly on the prevention and control of non-communicable diseases (NCDs). New York, 2011.
4. **World Health Organization.** Global health observatory (GHO) data. Available at: http://www.who.int/gho/ncd/mortality_morbidity/en/.
5. **World Health Organization.** Global report on diabetes. 2016.
6. **International Diabetes Federation.** IDF Diabetes Atlas. 7th ed., Brussels, Belgium, 2015.
7. **Van Belle TL, Coppieters KT, Von Herrath MG.** Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 2011. 91(1):79-118.
8. **Butalia S, Kaplan G, Khokhar B, Rabi D.** Environmental risk factors and type 1 diabetes: past, present, and future. *Can J Diabetes* 2016. 40(6):586-593.
9. **Alpert JS.** An amazing story: the discovery of insulin. *Am J Med* 2016. 129(3):231-232.
10. **Duan X, Mao S.** New strategies to improve the intranasal absorption of insulin. *Drug Discov Today* 2010. 15(11-12):416-427.
11. **Liu X, Li X, Zhang N, Zhao Z, Wen X.** Bioengineering strategies for the treatment of type I diabetes. *J Biomed Nanotechnol* 2016. 12(4):581-601.
12. **Qiu Y, Park K.** Environment-sensitive hydrogels for drug delivery. *Adv Drug Deliv Rev* 2001. 53(3):321-339.
13. **Sengupta D, Waldman SD, Li S.** From in vitro to in situ tissue engineering. *Ann Biomed Eng* 2014. 42(7):1537-1545.
14. **Kumar N, Desagani D, Chandran G, Ghosh NN, Karthikeyan G, Waigaonkar S, Ganguly A.** Biocompatible agarose-chitosan coated silver nanoparticle composite

for soft tissue engineering applications. *Artif Cells Nanomed Biotechnol* 2017. In press.
15. **Li S, Sengupta D, Chien S.** Vascular tissue engineering: from in vitro to in situ. *Wiley Interdiscip Rev Syst Biol Med* 2014. 6:61-76.
16. **Asghari F, Samiei M, Adibkia K, Akbarzadeh A, Davaran S.** Biodegradable and biocompatible polymers for tissue engineering application: a review. *Artif Cells Nanomed Biotechnol* 2016. 45(2):185-192.
17. **Lee EJ, Kasper FK, Mikos AG.** Biomaterials for tissue engineering. *Ann Biomed Eng* 2014. 42(2):323-327.
18. **Lu T, Li Y, Chen T.** Techniques for fabrication and construction of three-dimensional scaffolds for tissue engineering. *Int J Nanomedicine* 2013. 8:337-350.
19. **Liu, W, Thomopoulos S, Xia Y.** Electrospun nanofibers for regenerative medicine. *Adv Healthc Mater* 2012. 1(1):10-25.
20. **O'Connor R, McGuinness G.** Electrospun nanofibre bundles and yarns for tissue engineering applications: A review. *Proc Inst Mech Eng H* 2016. *230*(11):987-998.
21. **Triplett R, Budinskaya O.** New frontiers in biomaterials. *Oral Maxillofac Surg Clin North Am* 2017. 29(1):105-115.
22. **Miller J, Stevens K, Yang M, Baker B, Nguyen DH, Cohen D, Toro E, Chen A, Galie P, Yu X, et al.** Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater* 2012. 11(9):768-774.
23. **Gladman A, Matsumoto E, Nuzzo R, Mahadevan L, Lewis J.** Biomimetic 4D printing. *Nat Mater* 2016. 15(4):413-418.
24. **Khademhosseini A, Langer R.** A decade of progress in tissue engineering. *Nat Protoc* 2016. 11(10):1775-1781.
25. **Gasperini L, Mano J, Reis R.** Natural polymers for microencapsulation of cells. *J R Soc Interface* 2014. 11:20140817.
26. **Olabisi R.** Cell microencapsulation with synthetic polymers. *J Biomed Mater Res A* 2015. 103(2):846-859.
27. **Rogers J, Farney A, Al-Geizawi S, Iskandar S, Doares W, Gautreaux M, Hart L, Kaczmorski S, Reeves-Daniel A, Winfrey S, et al.** Pancreas transplantation: lessons learned from a decade of experience at Wake Forest Baptist Medical Center. *Rev Diabet Stud* 2011. 8(1):17-27.
28. **Ludwig B, Ludwig S, Steffen A, Saeger HD, Bornstein S.** Islet versus pancreas transplantation in type 1 diabetes: Competitive or complementary? *Curr Diab Rep* 2010. 10(6):506-511.
29. **Vaithilingam V, Tuch B.** Islet Transplantation and encapsulation: An update on recent developments. *Rev Diabet Stud* 2011. 8(1):51-67.
30. **Teramura Y, Iwata H.** Islet encapsulation with living cells for improvement of biocompatibility. *Biomaterials* 2009. 30(12):2270-2275.
31. **Montanya E.** Islet- and stem-cell-based tissue engineering in diabetes. *Curr Opin Biotechnol* 2004. 15(5):435-440.
32. **Williams S, Wang Q, MacGregor R, Siahaan T, Stehno-Bittel L, Berkland C.** Adhesion of pancreatic beta cells to biopolymer films. *Biopolymers* 2009. 91(8):676-685.
33. **Asti A, Gioglio L.** Natural and synthetic biodegradable polymers: different scaffolds for cell expansion and tissue formation. *Int J Artif Organs* 2014. 37(3):187-205.
34. **Lin C, Ki C, Shih H.** Thiol-norbornene photo-click hydrogels for tissue engineering applications. *J Appl Polym Sci* 2015. 132(8):41563.
35. **Makadia H, Siegel S.** Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 2011. 3(3):1377-1397.
36. **Gentile P, Chiono V, Carmagnola I, Hatton P.** An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. *Int J Mol Sci* 2014. 15(3):3640-3659.
37. **Ramshaw JA, Peng YY, Glattauer V, Werkmeister JA.** Collagens as biomaterials. *J Mater Sci: Mater Med* 2009. 20:S3-S8.
38. **Tedder M, Simionescu A, Chen J, Liao J, Simionescu D.** Assembly and testing of stem cell-seeded layered collagen constructs for heart valve tissue engineering. *Tissue Eng Part A* 2011. 17(1-2):25-36.
39. **Dunphy S, Bratt J, Akram K, Forsyth N, El Haj A.** Hydrogels for lung tissue engineering: Biomechanical properties of thin collagen-elastin constructs. *J Mech Behav Biomed Mater* 2014. 38:251-259.
40. **Zhou Y, Yao H, Wang J, Wang D, Liu Q, Li Z.** Greener synthesis of electrospun collagen/ hydroxyapatite composite fibers with an excellent microstructure for bone tissue engineering. *Int J Nanomedicine* 2015. 10:3203-3215.
41. **Jalili R, Rezakhanlou A, Hosseini-Tabatabaei A, Ao Z, Warnock G, Ghahary A.** Fibroblast populated collagen matrix promotes islet survival and reduces the number of islets required for diabetes reversal. *J Cell Physiol* 2011. 226(7):1813-1819.
42. **Xu J, Miao G, Zhao Y, Wei J.** Subcutaneous transplantation may not be an appropriate approach for the islets embedded in the collagen gel scaffolds. *Transplant Proc* 2011. 43(9):3205-3208.
43. **Ellis C, Suuronen E, Yeung T, Seeberger K, Korbutt G.** Bioengineering a highly vascularized matrix for the ectopic transplantation of islets. *Islets* 2013. 5(5):216-225.
44. **Yap W, Salvay D, Silliman M, Zhang X, Bannon Z, Kaufman D, Lowe W Jr, Shea L.** Collagen IV-modified scaffolds improve islet survival and function and reduce time to euglycemia. *Tissue Eng Part A* 2013. 19(21-22):2361-2372.
45. **Hoque ME, Nuge T, Yeow TK, Nordin N, Vara P.** Gelatin-based scaffolds for tissue engineering - a review. *Polym Res J* 2015. 9(1):15-32.
46. **Chen S, Zhang Q, Nakamoto T, Kawazoe N, Chen G.** Gelatin scaffolds with controlled pore structure and mechanical property for cartilage tissue engineering. *Tissue Eng Part C Methods* 2016. 22(3):189-198.
47. **Maji K, Dasgupta S, Pramanik K, Bissoyi A.** Preparation and evaluation of gelatin-chitosan-nanobioglass 3D porous scaffold for bone tissue engineering. *Int J Biomater* 2016. 2016:9825659.
48. **Han F, Dong Y, Su Z, Yin R, Song A, Li S.** Preparation, characteristics and assessment of a novel gelatin-chitosan sponge scaffold as skin tissue engineering material. *Int J Pharmaceut* 2014. 476(1-2):124-133.
49. **Kodama S, Kojima K, Furuta S, Chambers M, Paz A, Vacanti C.** Engineering functional islets from cultured cells. *Tissue Eng Part A* 2009. 15(11):3321-3329.
50. **Muthyala S, Bhonde RR, Nair PD.** Cytocompatibility studies of mouse pancreatic islets on gelatin-PVP semi IPN scaffolds in vitro. *Islets* 2010. 2(6):357-366.
51. **Muthyala S, Rana RV, Mohanty M, Mohanan PV, Nair P.** The reversal of diabetes in rat model using mouse insulin producing cells - a combination approach of tissue engineering and microencapsulation. *Acta Biomater* 2011.

7(5):2153-2162.
52. **Aloysious N, Nair PD.** Enhanced survival and function of islet-like clusters differentiated from adipose stem cells on a three-dimensional natural polymeric scaffold: an in vitro study. *Tissue Eng Part A* 2014. 20(9-10):1508-1522.
53. **Janmey P, Winer J, Weisel J.** Fibrin gels and their clinical and bioengineering applications. *J R Soc Interface* 2009. 6(30):1-10.
54. **Yasuda H, Kuroda S, Shichinohe H, Kamei S, Kawamura R, Iwasaki Y.** Effect of biodegradable fibrin scaffold on survival, migration, and differentiation of transplanted bone marrow stromal cells after cortical injury in rats. *J Neurosurg* 2010. 112(2):336-344.
55. **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(1):34-44.
56. **Niknamasl A, Ostad SN, Soleimani M, Azami M, Salmani MK, Lotfibakhshaiesh N, Ebrahimi-Barough S, Karimi R, Roozafzoon R, Ai J.** A new approach for pancreatic tissue engineering: human endometrial stem cells encapsulated in fibrin gel can differentiate to pancreatic islet beta-cell. *Cell Biol Int* 2014. 38(10):1-9.
57. **Khorsandi L, Nejad-Dehbashi F, Ahangarpour A, Hashemitabar M.** Three-dimensional differentiation of bone marrow-derived mesenchymal stem cells into insulin-producing cells. *Tissue Cell* 2015. 47(1):66-72.
58. **Riopel M, Stuart W, Wan R.** Fibrin improves beta (INS-1) cell function, proliferation and survival through integrin avb3. *Acta Biomater* 2013. 9(9):8140-8148.
59. **Kim JS, Lim JH, Nam HY, Lim HJ, Shin JS, Shin JY, Ryu JH, Kim K, Kwon IC, Jin SM, et al.** In situ application of hydrogel-type fibrin-islet composite optimized for rapid glycemic control by subcutaneous xenogeneic porcine islet transplantation. *J Control Release* 2012. 162(2):382-390.
60. **Kohane DS, Langer R.** Polymeric biomaterials in tissue engineering. *Pediatr Res* 2008. 63(5):487-491.
61. **Varoni E, Tschon M, Palazzo B, Nitti P, Martini L, Rimondini L.** Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. *Connect Tissue Res* 2012. 53(6):548-554.
62. **Bhat S, Tripathi A, Kumar A.** Supermacroporous chitosan-agarose-gelatin cryogels: in vitro characterization and in vivo assessment for cartilage tissue engineering. *J R Soc Interface* 2011. 8(57):540-554.
63. **Tripathi A, Melo JS.** Preparation of a sponge-like biocomposite agarose–chitosan scaffold with primary hepatocytes for establishing an in vitro 3D liver tissue model. *RSC Adv* 2015. 5(39):30701-30710.
64. **Gazda LS, Vinerean HV, Laramore MA, Hall RD, Carraway JW, Smith BH.** Pravastatin improves glucose regulation and biocompatibility of agarose encapsulated porcine islets following transplantation into pancreatectomized dogs. *J Diabetes Res* 2014. 2014:405362.
65. **Luan NM, Iwata H.** Long-term allogeneic islet graft survival in prevascularized subcutaneous sites without immunosuppressive treatment. *Am J Transplant* 2014. 14:1533-1542.
66. **Hilderink J, Spijker S, Carlotti F, Lange L, Engelse M, Van Blitterswijk C, De Koning E, Karperien M, Apeldoorn A.** Controlled aggregation of primary human pancreatic islet cells leads to glucose-responsive pseudo islets comparable to native islets. *J Cell Mol Med* 2015. 19(8):1836-1846.
67. **Ichihara Y, Utoh R, Yamada M, Shimizu T, Uchigata Y.** Size effect of engineered islets prepared using microfabricated wells on islet cell function and arrangement. *Heliyon* 2016. 2(6):e00129.
68. **Kim JW, Vang S, Luo JH, Luo L.** Human islet co-cultured with bone marrow mesenchymal stem cells in 3D scaffolding may augment pancreatic beta cell function. *J Biomater Tissue Eng* 2017. 7(3):203-209.
69. **Calafiore R, Basta G.** Clinical application of microencapsulated islets: actual prospectives on progress and challenges. *Adv Drug Deliv Rev* 2014. 67-68:84-92.
70. **Haitao Z, Liang Y, Yayi H, Yi L, Bo W.** Microencapsulated pig islet xenotransplantation as an alternative treatment of diabetes. *Tissue Eng Part B Rev* 2015. 21(5):474-489.
71. **Steele JA, Halle JP, Poncelet D, Neufeld RJ.** Therapeutic cell encapsulation techniques and applications in diabetes. *Adv Drug Deliv Rev* 2014. 67-68:74-83.
72. **Lim F, Sun AM.** Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980. 210(4472):908-910.
73. **Andersen T, Auk-Emblem P, Dornish M.** 3D cell culture in alginate hydrogels. *Microarrays* 2015. 4(2):133-161.
74. **Opara EC, Mirmalek-Sani SH, Khanna O, Moya ML, Brey EM.** Design of a bioartificial pancreas. *Investig Med* 2010. 58(7):831-837.
75. **Hall KK, Gattas-Asfura KM, Stabler CL.** Microencapsulation of islets within alginate/poly(ethylene glycol) gels cross-linked via Staudinger ligation. *Acta Biomater* 2011. 7(2):614-624.
76. **Pareta R, McQuilling JP, Sittadjody S, Jenkins R, Bowden S, Orlando G, Farney AC, Brey E M, Opara EC.** Long-term function of islets encapsulated in a redesigned alginate microcapsule construct in omentum pouches of immune-competent diabetic rats. *Pancreas* 2014. 43(4):605-613.
77. **Richardson T, Kumta PN, Banerjee I.** Alginate encapsulation of human embryonic stem cells to enhance directed differentiation to pancreatic islet-like cells. *Tissue Eng Part A* 2014. 20(23-24):3198-3211.
78. **Marchioli G, Van Gurp L, Van Krieken PP, Stamatialis D, Engelse M, van Blitterswijk C A, Karperien MB, de Koning E, Alblas J, Moroni L.** Fabrication of three-dimensional bioplotted hydrogel scaffolds for islets of Langerhans transplantation. *Biofabrication* 2015. 7(2):025009.
79. **Vegas AJ, Veiseh O, Gürtler M, Millman JR, Pagliuca FW, Bader AR, Doloff J, Li J, Chen M, Olejnik K, et al.** Long-term glycemic control using polymer encapsulated, human stem-cell derived beta-cells in immune competent mice. *Nat Med* 2016. 22(3):306-311.
80. **Edgar L, McNamara K, Wong T, Tamburrini R, Katari R, Orlando G.** Heterogeneity of scaffold biomaterials in tissue engineering. *Materials* 2016. 9(5):332.
81. **Kundu B, Rajkhowa R, Kundu SC, Wang X.** Silk fibroin biomaterials for tissue regenerations. *Adv Drug Deliv Rev* 2013. 65:457-470.
82. **Kearns V, MacIntosh AC, Crawford A, Hatton PV.** Silk-based biomaterials for tissue engineering. In: Ashammakhi N, Reis R, Chiellini F (eds.). Topics in tissue engineering. Oulu University, 2008, chapter 1, pp. 1-19.
83. **Kanitkar M, Kale VP.** Stem cells and extra cellular matrices: applications in tissue engineering. *Biomed Res J* 2014. 1(2):95-107.
84. **Shao W, He J, Sang F, Ding B, Chen L, Cui S, Li K, Han Q, Tan W.** Coaxial electrospun aligned tussah silk fib-

roin nanostructured fiber scaffolds embedded with hydroxyapatite–tussah silk fibroin nanoparticles for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2016. 58:342-351.

85. **Naghashzargar E, Fare S, Catto V, Bertoldi S, Semnani D, Karbasi S, Maria CT.** Nano/ micro hybrid scaffold of PCL or P3HB nanofibers combined with silk fibroin for tendon and ligament tissue engineering. *J Appl Biomater Funct Mater* 2015. 13(2):e156-168.
86. **Wei G, Li C, Fu Q, Xu Y, Li H.** Preparation of PCL/silk fibroin/collagen electrospun fiber for urethral reconstruction. *Int Urol Nephrol* 2015. 47(1):95-99.
87. **Singh YP, Bhardwaj N, Mandal BB.** Potential of agarose/silk fibroin blended hydrogel for in vitro cartilage tissue engineering. *ACS Appl Mater Interfaces* 2016. 8(33):21236-21249.
88. **Davis NE, Beenken-Rothkopf LN, Mirsoian A, Kojic N, Kaplan D, Barron AE, Fontaine MJ.** Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk hydrogel. *Biomaterials* 2012. 33(28):6691-6697.
89. **Do SG, Park JH, Nam H, Kim JB, Lee JY, Oh YS, Suh JY.** Silk fibroin hydrolysate exerts an anti-diabetic effect by increasing pancreatic beta-cell mass in C57BL/KsJ-db/db mice. *J Vet Sci* 2012. 13(4):339-344.
90. **Hamilton DC, Shih HH, Schubert RA, Michie SA, Staats PN, Kaplan DL, Fontaine MJ.** A silk-based encapsulation platform for pancreatic islet transplantation improves islet function in vivo. *J Tissue Eng Regen Med* 2015. 11(3):887-895.
91. **Kumar M, Nandi SK, Kaplan DL, Mandal BB.** Localized immunomodulatory silk macrocapsules for islet-like spheroid formation and sustained insulin production. *ACS Biomater Sci Eng* 2017. 3(10):2443-2456.
92. **Athanasiou K.** Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* 1996. 17(2):93-102.
93. **Chun S, Huang Y, Xie W, Hou Y, Huang R, Song C.** Adhesive growth of pancreatic islet cells on a polyglycolic acid fibrous scaffold. *Transplant Proc* 2008. 40(5):1658-1663.
94. **Nagato H, Umebayashi Y, Wako M, Tabata Y, Manabe M.** Collagen-poly glycolic acid hybrid matrix with basic fibroblast growth factor accelerated angiogenesis and granulation tissue formation in diabetic mice. *J Dermatol* 2006. 33(10):670-675.
95. **Song C, Huang Y, Wei Z, Hou Y, Xie W, Huang R, Song YM, Lv HG, Song CF.** Polyglycolic acid-islet grafts improve blood glucose and insulin concentrations in rats with induced diabetes. *Transplant Proc* 2009. 41(5):1789-1793.
96. **Li Y, Fan P, Ding X, Tian X, Feng X, Yan H, Pan XM, Tian PX, Zhen J, Ding CG, et al.** Polyglycolic acid fibrous scaffold improving endothelial cell coating and vascularization of islet. *Chin Med J* 2017. 130(7):832-839.
97. **Fonte P, Araujo F, Silva C, Pereira C, Reis S, Santos HA, Sarmento B.** Polymer-based nanoparticles for oral insulin delivery: revisited approaches. *Biotechnol Adv* 2015. 33(6):1342-1354.
98. **Sabek OM, Farina M, Fraga DW, Afshar S, Ballerini A, Filgueira CS, Thekkedath UR, Grattoni A, Gaber AO.** Three-dimensional printed polymeric system to encapsulate human mesenchymal stem cells differentiated into islet-like insulin-producing aggregates for diabetes treatment. *J Tissue Eng* 2016. 7:2041731416638198.
99. **Farina M, Ballerini A, Fraga D, Hogan M, Nicolov E, Demarchi D, Scaglione F, Sabek O, Horner P, Thekkedath U, et al.** 3D printed vascularized device for subcutaneous transplantation of human islets. *Biotechnol J* 2017. 12(9):1700169.
100. **Tyler B, Gullotti D, Mangraviti A, Utsuki T, Brem H.** Polylactic acid (PLA) controlled delivery carriers for biomedical applications. *Adv Drug Deliv Rev* 2016. 107:163-175.
101. **Li H, Li B, Wang Q, Xiao Y, Chen XM, Li W.** Attenuation of inflammatory response by 25-hydroxyvitamin D3-loaded polylactic acid microspheres in treatment of periodontitis in diabetic rats. *Chin J Dent Res* 2013. 17(2):91-98.
102. **Tomar L, Tyagi C, Kumar M, Kumar P, Singh H, Choonara YE, Pillay V.** In vivo evaluation of a conjugated poly (lactide-ethylene glycol) nanoparticle depot formulation for prolonged insulin delivery in the diabetic rabbit model. *Int J Nanomedicine* 2013. 8:505-520.
103. **Kasoju N, Kubies D, Fabryova E, Kriz J, Kumorek MM, Sticova E, Rypacek F.** In vivo vascularization of anisotropic channeled porous polylactide-based capsules for islet transplantation: the effects of scaffold architecture and implantation site. *Physiol Res* 2015. 64:S75.
104. **Pan Z, Ding J.** Poly (lactide-co-glycolide) porous scaffolds for tissue engineering and regenerative medicine. *Interface focus* 2012. 2(3):366-377.
105. **Zhao W, Li J, Jin K, Liu W, Qiu X, Li C.** Fabrication of functional PLGA-based electrospun scaffolds and their applications in biomedical engineering. *Mater Sci Eng C Mater Biol Appl* 2016. 59:1181-1194.
106. **Mironov AV, Grigoryev AM, Krotova LI, Skaletsky NN, Popov VK, Sevastianov VI.** 3D printing of PLGA scaffolds for tissue engineering. *J Biomed Mater Res A* 2017. 105(1):104-109.
107. **Blomeier H, Zhang X, Rives C, Brissova M, Hughes E, Baker M, Powers AC, Kaufman DB, Shea LD, Lowe WL Jr.** Polymer scaffolds as synthetic microenvironments for extrahepatic islet transplantation. *Transplantation* 2006. 82(4):452.
108. **Salvay DM, Rives CB, Zhang X, Chen F, Kaufman DB, Lowe WL Jr, Shea LD.** Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation. *Transplantation* 2008. 85(10):1456.
109. **Daoud JT, Petropavlovskaia MS, Patapas JM, Degrandpre CE, DiRaddo RW, Rosenberg L, Tabrizian M.** Long-term in vitro human pancreatic islet culture using three-dimensional microfabricated scaffolds. *Biomaterials* 2011. 32(6):1536-1542.
110. **Kheradmand T, Wang S, Gibly RF, Zhang X, Holland S, Tasch J, Graham JG, Kaufman DB, Miller SD, Shea LD, Luo X.** Permanent protection of PLG scaffold transplanted allogeneic islet grafts in diabetic mice treated with ECDI-fixed donor splenocyte infusions. *Biomaterials* 2011. 32(20):4517-4524.
111. **Pasek RC, Kavanaugh TE, Duvall CL, Gannon MA.** Sustained administration of beta-cell mitogens to intact mouse islets ex vivo using biodegradable poly (lactic-co-glycolic acid) microspheres. *J Vis Exp* 2016. (117):e54664.
112. **Liu L, Tan J, Li B, Xie Q, Sun J, Pu H, Zhang L.** Construction of functional pancreatic artificial islet tissue composed of fibroblast-modified polylactic-co-glycolic acid membrane and pancreatic stem cells. *J Biomater Appl* 2017.

32(3):362-372.
113. **Mkhabela VJ, Ray SS.** Poly (epsilon-caprolactone) nanocomposite scaffolds for tissue engineering: a brief overview. *J Nanosci Nanotechnol* 2014. 14(1):535-545.
114. **Hoveizi E, Khodadadi S, Tavakol S, Karima O, Nasiri-Khalili MA.** Small molecules differentiate definitive endoderm from human induced pluripotent stem cells on PCL scaffold. *Appl Biochem Biotechnol* 2014. 173(7):1727-1736.
115. **Bajpai SK, Chand N, Soni S.** Controlled release of antidiabetic drug Gliclazide from poly (caprolactone)/poly (acrylic acid) hydrogels. *J Biomater Sci Polym Ed* 2015. 26(14):947-962.
116. **Gholipour-Kanani A, Bahrami SH, Rabbani S.** Effect of novel blend nanofibrous scaffolds on diabetic wounds healing. *IET Nanobiotechnol* 2016. 10(1):1-7.
117. **Ranjbar-Mohammadi M, Rabbani S, Bahrami SH, Joghataei MT, Moayer F.** Antibacterial performance and in vivo diabetic wound healing of curcumin loaded gum tragacanth/poly (-caprolactone) electrospun nanofibers. *Mater Sci Eng C Mater Biol Appl* 2016. 69:1183-1191.
118. **Marchioli G, Luca AD, de Koning E, Engelse M, Van Blitterswijk CA, Karperien M, Van Apeldoorn AA, Moroni L.** Hybrid polycaprolactone/alginate scaffolds functionalized with VEGF to promote de novo vessel formation for the transplantation of islets of Langerhans. *Adv Healthc Mater* 2016. 5(13):1606-1616.
119. **Smink AM, Hertsig DT, Schwab L, van Apeldoorn AA, de Koning E, Faas MM, de Haan BJ, de Vos P.** A retrievable, efficacious polymeric scaffold for subcutaneous transplantation of rat pancreatic islets. *Ann Surg* 2017. 266(1):149-157.
120. **Pedraza E, Brady AC, Fraker CA, Stabler CL.** Synthesis of macroporous poly (dimethylsiloxane) scaffolds for tissue engineering applications. *J Biomater Sci Polym Ed* 2013. 24(9):1041-1056.
121. **Pedraza E, Brady AC, Fraker CA, Molano RD, Sukert S, Berman DM, Kenyon NS, Pileggi A, Ricordi C, Stabler CL.** Macroporous three-dimensional PDMS scaffolds for extrahepatic islet transplantation. *Cell Transplant* 2013. 22(7):1123-1135.
122. **Brady AC, Martino MM, Pedraza E, Sukert S, Pileggi A, Ricordi C, Hubbell JA, Stabler CL.** Proangiogenic hydrogels within macroporous scaffolds enhance islet engraftment in an extrahepatic site. *Tissue Eng Part A* 2013. 19(23-24):2544-2552.
123. **Jiang K, Weaver JD, Li Y, Chen X, Liang J, Stabler CL.** Local release of dexamethasone from macroporous scaffolds accelerates islet transplant engraftment by promotion of anti-inflammatory M2 macrophages. *Biomaterials* 2017. 114:71-81.
124. **Zawalich WS, Tesz GJ, Yamazaki H, Zawalich KC, Philbrick W.** Dexamethasone suppresses phospholipase C activation and insulin secretion from isolated rat islets. *Metabolism* 2006. 55(1):35-42.
125. **Frei AW, Li Y, Jiang K, Buchwald P, Stabler CL.** Local delivery of fingolimod from 3-D scaffolds impacts islet graft efficacy and microenvironment in a murine diabetic model. *J Tissue Eng Regen Med* 2017. 1(12):393-404.
126. **Kozlovskaya V, Zavgorodnya O, Kharlampieva E.** Encapsulation and surface engineering of pancreatic islets: advances and challenges. In: Lin C. Biomedicine. InTech, 2012, chapter 1, pp. 3-35.
127. **Mason MN, Arnold CA, Mahoney MJ.** Entrapped collagen type 1 promotes differentiation of embryonic pancreatic precursor cells into glucose-responsive beta-cells when cultured in three-dimensional PEG hydrogels. *Tissue Eng Part A* 2009. 15(12):3799-3808.
128. **Mason MN, Mahoney MJ.** A novel composite construct increases the vascularization potential of PEG hydrogels through the incorporation of large fibrin ribbons. *J Biomed Mater Res A* 2010. 95(1):283-293.
129. **Kizilel S, Scavone A, Liu X, Nothias JM, Ostrega D, Witkowski P, Millis M.** Encapsulation of pancreatic islets within nano-thin functional polyethylene glycol coatings for enhanced insulin secretion. *Tissue Eng Part A* 2010. 16(7):2217-2228.
130. **De Carlo E, Baiguera S, Conconi MT, Vigolo S, Grandi C, Lora S, Martini C, Maffei P, Tamagno G, Vettor R, et al.** Pancreatic acellular matrix supports islet survival and function in a synthetic tubular device: in vitro and in vivo studies. *Int J Mol Med* 2010. 25(2):195-202.
131. **Bernard AB, Lin CC, Anseth KS.** A microwell cell culture platform for the aggregation of pancreatic beta-cells. *Tissue Eng Part C Methods* 2012. 18(8):583-592.
132. **Chae SY, Kim SW, Bae YH.** Effect of cross-linked hemoglobin on functionality and viability of microencapsulated pancreatic islets. *Tissue Eng* 2002. 8(3):379-394.
133. **Nadithe V, Mishra D, Bae YH.** Poly (ethylene glycol) cross-linked hemoglobin with antioxidant enzymes protects pancreatic islets from hypoxic and free radical stress and extends islet functionality. *Biotechnol Bioeng* 2012. 109(9):2392-2401.
134. **Golab K, Kizilel S, Bal T, Hara M, Zielinski M, Grose R, Savari O, Wang XJ, Wang LJ, Tibudan M, et al.** Improved coating of pancreatic islets with regulatory T cells to create local immunosuppression by using the biotin-polyethylene glycol-succinimidyl valeric acid ester molecule. *Transplant Proc* 2014. 46(6):1967-1971.
135. **Bal T, Nazli C, Okcu A, Duruksu G, Karaöz E, Kizilel S.** Mesenchymal stem cells and ligand incorporation in biomimetic poly (ethylene glycol) hydrogels significantly improve insulin secretion from pancreatic islets. *J Tissue Eng Regen Med* 2017. 11(3):694-703.
136. **Ballian N, Brunicardi FC.** Islet vasculature as a regulator of endocrine pancreas function. *World J Surg* 2007. 31(4):705-714.
137. **Phelps EA, Templeman KL, Thule PM, Garcia AJ.** Engineered VEGF-releasing PEG-MAL hydrogel for pancreatic islet vascularization. *Drug Deliv Transl Res* 2015. 5(2):125-136.
138. **Rios PD, Zhang X, Luo X, Shea LD.** Mold-casted non-degradable, islet macro-encapsulating hydrogel devices for restoration of normoglycemia in diabetic mice. *Biotechnol Bioeng* 2016. 113(11):2485-2495.
139. **Pandol SJ.** The exocrine pancreas. In: Granger DN, Granger JP. Colloquium series on integrated systems physiology: from molecule to function. Morgan and Claypool Life Sciences, 2011, vol. 3, pp. 1-64.
140. **Brereton MF, Vergari E, Zhang Q, Clark A.** Alpha-, delta-and PP-cells: are they the architectural cornerstones of islet structure and co-ordination? *J Histochem Cytochem* 2015. 63(8):575-891.
141. **O'Sullivan ES, Johnson AS, Omer A, Hollister-Lock J, Bonner-Weir S, Colton CK, Weir GC.** Rat islet cell aggregates are superior to islets for transplantation in microcapsules. *Diabetologia* 2010. 53(5):937-945.

142. **Luo JZ, Xiong F, Al-Homsi AS, Ricordi C, Luo L.** Allogeneic bone marrow co-cultured with human islets significantly improves islet survival and function in vivo. *Transplantation* 2013. 95(6):801.
143. **Zheng X, Wang X, Ma Z, Sunkari VG, Botusan I, Takeda T, Björklund A, Inoue M, Catrina SB, Brismar K, et al.** Acute hypoxia induces apoptosis of pancreatic beta-cell by activation of the unfolded protein response and upregulation of CHOP. *Cell Death Dis* 2012. 3(6):e322.
144. **Wang P, Schuetz C, Ross A, Dai G, Markmann JF, Moore A.** Immune rejection after pancreatic islet cell transplantation: in vivo dual contrast-enhanced MR imaging in a mouse model. *Radiology* 2013. 266(3):822-830.