# Chapter 7
# Differentiation of Stem Cells into Pancreatic Lineage: In vitro Cell Culture, in vivo Transplantation in Animal Models

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**Abstract** The pancreas is an abdominal glandular organ which is involved in the maintenance of the nutritional balance, through the synthesis and secretion of hormones and enzymes. It consists of two main parts: endocrine and exocrine glands. Endocrine part of the pancreas is composed of clusters of cells which are collectively called as islets of Langerhans, containing five types of cells; these cells produce different hormones that are responsible for maintaining the balance of glucose in blood. Exocrine part of the pancreas consists of acinar cells and ductal cells. They are involved in the secretion of enzymes that assist digestion. The absence of proper functioning β-cells causes diabetes. Diabetes mellitus is a chronic metabolic disorder characterized by deficiency or loss of the insulin-producing β-cells of the pancreas. Stem cells are a revolution in modern medicine and have become the most promising therapeutic approach for treating diabetes that can offer an alternative source of insulin-producing cells. This chapter reviews some attempts that have been used depicting different differentiation methodologies of transforming stem cells into β-cells in vitro and in vivo. It also sheds light on some of the human clinical trials, and the results used for stem cells for diabetes treatment that have been achieved.

**Keywords** Exocrine · Endocrine · Islets of Langerhans · Ductal cells · Differentiation · Stem cells · Insulin

## Pancreas

The pancreas is a glandular organ, located in the upper abdomen behind the stomach (Githens, 1994). The pancreas anatomy is an irregular shape and classified into four main parts: head, neck, body, and tail (Frantz et al., 2012). It has a significant function in controlling blood glucose homeostasis by producing digestive enzymes and hormones. It consists of two major compartments: exocrine and endocrine parts of pancreas. The exocrine tissue forms up to 90% of the pancreas which contain acinar

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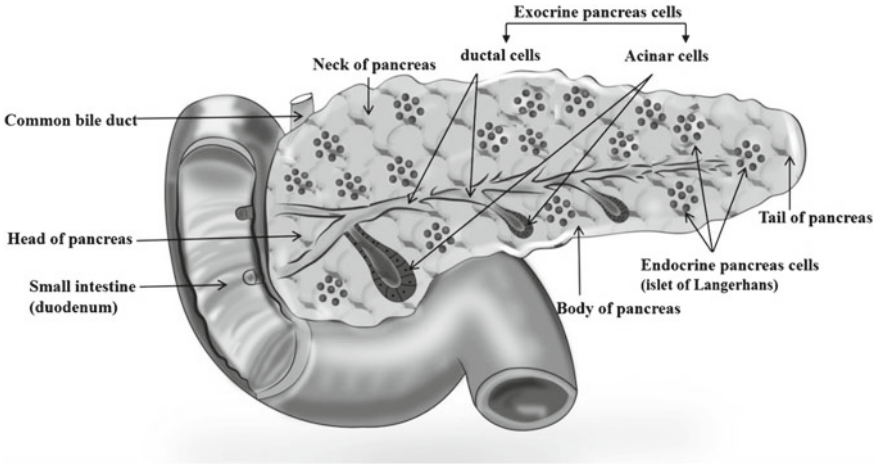


**Fig. 7.1** Structure of the pancreas. The anatomy of pancreas is divided into four parts: head, neck, body, and tail. Pancreas consist of two main sections: exocrine and endocrine cells, each performs different functions. Exocrine cells consist of acinar cells and ductal cells. Endocrine cells are clustered into the islets of Langerhans

cells and ductal cells that produce various digestive enzymes into the digestive tract. The endocrine tissue forms up to 10% of the entire pancreas. It synthesizes and produces hormones that participate in regulating the metabolism of glucose, carbohydrates, lipid, and protein. The endocrine cells are located in groups of cells called the islets of Langerhans. The islets of Langerhans consist of different cell types that produce different hormones including α-(glucagon), β-(insulin), δ-(somatostatin), ε-(ghrelin), and PP-cells (pancreatic polypeptide) (Bastidas-Ponce et al., 2017; Cabrera et al., 2006). A simple illustration of the pancreas structure is given in Fig. 7.1.

## Pancreas Development

### *Development Pancreas in Embryonic Stage and Endoderm Specification*

During the early embryonic development stage of gastrulation, three primary germ layers, i.e., ectoderm, mesoderm, and endoderm are formed. These layers were developed from intensive cell migration of the inner cell mass. These three germ layers differentiate into specific tissues and organs during embryonic development. The gastrointestinal organs and the pancreas are originated from the endoderm. An epithelial sheet of cells of definitive endoderm (DE) formation begins between embryonic day (E) 6.5–7.5 in mice. The (DE) is defined as a group of multi-functional stem

cells that are allocated as a single germ layer occurred during gastrulation. Endoderm patterning process is regulated interactions with surrounding mesoderm tissues (Guo & Hebrok, 2009; Zorn & Wells, 2009). Development of pancreas is a dynamic activity which is controlled by a multifaceted regulatory network consisting of various transcription factors and signaling molecules, critical for growth and development of the pancreas in early stages. The wingless-type MMTV integration site (Wnt) signaling pathway and the transforming growth factor beta (TGF-β) are important for the generation of definitive endoderm during gastrulation (D'Amour et al., 2005). Studies show that effect the deleting β-catenin can be decreased the numbering of β-cell in pancreas. This is attributed to the defect in multipotent progenitor cell expansion rather than the subsequent differentiation steps (Bastidas-Ponce et al., 2017). Wnt signaling regulates the pancreatic progenitor cells proliferation, β-cell, and acinar cell replication (Guo & Hebrok, 2009). Another important signaling in this stage is a member of the TGF-B family called nodal. Nodal signaling is essential for determining the anterior/posterior axis (D'Amour et al., 2005). It is also necessary for the starting of gastrulation through regulation of Wnt signaling, fibroblast growth factors (FGFs). Nodal signaling induces the endoderm transcription factors expression such as Sox17, GATA4, and GATA6 (Zorn & Wells, 2009).

## *Specification of the Dorsal and Ventral Pancreas*

The first pancreatic specification begins at embryonic day E8.5 in mice and third week after the fertilization in humans. After gastrulation, the pancreas originates as two distinct buds in the dorsal and ventral pancreatic buds foregut (Watt et al., 2007). In mice, the first appearance of the dorsal pancreatic bud is at E9.0, while the ventral bud develops at E9.5. This distinction is caused by different signals from nearby tissues derived from the mesoderm. The dorsal bud produces the gastric and splenic lobes, and the ventral bud develops the lobe that runs along the proximal duodenum in the mouse (Dassaye et al., 2016; Watt et al., 2007). The main part of the head, body, and tail of the developed pancreas was generated by the dorsal bud, whereas the ventral bud participates in the inferior part of the head organ in humans (Piper et al., 2004). The dorsal bud develops adjacent to the notochord, composed of cells originating from the mesoderm. At E11.5 in mice, the ductal epithelium produces and branches into the nearby mesenchyme; giving rise to highly branched structures (Zhou et al., 2007). There are several important signaling pathways involved in pancreas development and formation of the dorsal bud including FGFs, TGF-B, retinoic acid, Sonic hedgehog (Shh), Wnt, and Notch.

## *The Primary Transition*

The pancreatic development is divided into three main stages: The primary transition is characterized by active proliferation of multipotent pancreatic progenitor cells that generate a stratified epithelium, with microlumen formation that are fused at the end to develop (Pictet et al., 1972). The first wave of insulin, glucagon, and double positive cells show in the dorsal bud of mice at this phase (Herrera, 2000). The primary transition in humans forms a proto-differentiated epithelium (Sarkar et al., 2008). In different transition stages, transcription factors (extrinsic and intrinsic signals) play essential roles during pancreatic development that includes the initiation of transcription, regulating pancreatic morphogenesis, controlling cell differentiation, and maintenance of the cellular phenotypes and function (Dassaye et al., 2016). Transcription factors involved in the primary transition stage are summarized below.

### Pancreatic Duodenal Homeobox Gene 1 (Pdx1)

Pdx1 is a homeodomain transcription factor that has a crucial regulator function in normal pancreatic development, β-cell survival and function. It is called as insulin promoter factor 1 (IPF1). The expression of Pdx1 appears early in the dorsal and ventral buds development at E8.5 (Ahlgren et al., 1996). It is considered as an essential entry point to the dissected complex signaling and transcriptional regulatory networks which plays critical roles in proliferation and differentiation of the pancreas (Pan & Wright, 2011). Pdx1 is expressed in all cell development from endoderm-endocrine, exocrine and ductal (Dassaye et al., 2016). By lineage, tracing methods show that early Pdx1 positive cells produce both exocrine and endocrine components (Gannon et al., 2000). In mice, the expression of Pdx1 is limited to β-cells by E15.5, as it controls the insulin gene expression in the β-cells (Stoffers et al., 1997). In humans, the expression of Pdx1 occurs approximately on the 7th week of gestation and continues until the mature β-cell is formed (Kaneto & Matsuoka, 2015). Both humans and rodents, the whole deficiency of Pdx1 expression leads to pancreatic agenesis, while lack Pdx1expression causes dysfunction of β-cell, exponential death of β-cell, and eventually diabetes (Fujimoto & Polonsky, 2009).

### Pancreas Specific Transcription Factor 1a (Ptf1a)

Ptf1a is a heterotrimeric of the basic helix-loop-helix (bHLH) transcription factor composing of p48 subunit (Beres et al., 2006). It plays a serious role for the dorsal pancreatic endoderm development and exocrine gene transcription (Yoshitomi & Zaret, 2004). During development of pancreas, the Ptf1a expression level determines the cell fate. Low expression determines the endocrine cell fate while the high level of Ptf1a promotes the exocrine and inhibits the endocrine cell fate (Dassaye et al.,

2016). Lineage tracing study showed that Ptf1a is expressed in pancreatic progenitors, parallel with Pdx1, and its expression becomes gradually limited to the exocrine cells by E13.5, whereas it regulated enzyme release in exocrine tissue (Kawaguchi et al., 2002). In addition, Ptf1a is essential for Notch signaling mediated control in early development of pancreas by regulating the delta-like ligand (DII1) expression (Ahnfelt-Rønne et al., 2012). Recessive Ptf1a mutations in humans generate isolated neonatal diabetes mellitus (NDM), and this type of diabetes is associated with cerebral agenesis (Sellick et al., 2004).

**Hepatocyte Nuclear Factor 1 Beta (Hnf1b)**

Hnf1b is a nuclear transcription factor of the homeodomain family. It has a serious role in the specification of endocrine and exocrine cell fate and the endocrine precursors generation (De Vas et al., 2015). In the mice model, Hnf1b was first detected in the primitive endoderm on E4.5 and which is required for specification of the primitive endoderm lineage. Hnf1b is expressed in the early pancreatic development. It has a role in the precursor neurogenin 3 (Ngn3) positive cells which are destined to become islet cells (Maestro et al., 2007). Moreover, Hnf1b acts as a key player in early pancreas development. The expression of Hnf1b showed in the pre-pancreatic foregut endoderm and in initial pancreatic progenitor cells (Lau et al., 2018). Knockout mouse models for Hnf1b have both exocrine and endocrine defects, which serves as evidence for the importance of the Hnf1b factor in early development and specification (Haumaitre et al., 2005).

**GATA Binding Protein 4 and 6 (GATA 4) and (GATA 6)**

GATA 4 and 6 are zinc finger transcription factor family members; both are associated with the early stages of pancreatic development (Decker et al., 2006). Both have been co-expressed during initial foregut endoderm, later the dorsal and ventral pancreatic buds epithelial. GATA4 expression in acinar differentiated cells (Ketola et al., 2004). GATA6 expression is limited to endocrine and ductal cells, and it is required for the mature acinar cell maintenance (Decker et al., 2006). The ventral pancreatic is not formed in null GATA4 mice, while the dorsal pancreatic is formed normally (Watt et al., 2007). Moreover, GATA4 mutations caused congenital heart defects in humans. GATA6 haploinsufficiency mutations in humans cause pancreatic agenesis and cardiac abnormalities (Chao et al., 2008).

**SRY Sex Determining Region Y Box 17 (Sox17)**

Sox17 is a high mobility group (HMG) box transcription factor, which controls endoderm organ formation. It is required to maintain boundaries of the biliary system

between the liver and the ventral pancreas (Spence et al., 2009). It acts as a transcriptional regulator for other essential transcription factors during endoderm development, including Hnf1b, which is known to regulate postnatal β-cell function. Additionally, Sox17 is involved in insulin production and traffic regulation in β-cells in normal and diabetic conditions (Jonatan et al., 2014).

#### Sex Determining Region Y Box 9 (Sox9)

Sox9 is another HMG box transcription factor that is co-expressed with Pdx1 in multipotent progenitor cells (MPCs) at E9.5–12.5. Sox9 has an essential function in the formation of the pancreatic lineages that include ductal, islet and acinar lineages by stimulating their proliferation, persistence, and survival under an undifferentiated condition (Seymour et al., 2007). The inactivation of Sox9 expression caused hypoplasia of the dorsal and ventral buds in mice (Akiyama et al., 2005). The deficiency of Sox9 in mice leads to a failure in development of pancreas and this resulting in death. Positive cells of Sox9 in humans produce mature endocrine cells; however, Sox9 expression is limited to ductal cells in the mature pancreas (Seymour et al., 2007). Also, Sox9 is important for maintaining the identity of MPCs through a process that can be related to FGF signals of mesenchymal cells (Seymour et al., 2012).

It has a role in maintenance of multipotent progenitors by regulator Hnf1b and other factors (Lynn et al., 2007).

#### Insulin Gene Enhancer Binding Protein (Isl-1)

Isl1 is a LIM-homeodomain (LIM-HD) transcription factor belonging to the most important subfamilies of homeobox genes (Wang et al., 2014). It has a regulating role in pancreas primary and secondary transitions which is involved in dorsal pancreatic mesenchyme development in primary transitions, while secondary transition is participated in all endocrine cell and dorsal exocrine and formation (Dassaye et al., 2016). In the dorsal bud, and the mesenchyme surrounded the dorsal bud Isl1 was expressed. Isl1 expression is essential for the formation of the dorsal bud and differentiation of insulin-producing cells (Ahlgren et al., 1997). It is also expressed in all pancreatic islet cells. Its deficiency not only decreases islet cell proliferation but also leads to the continued loss of islet cells in mice pancreas (Dassaye et al., 2016).

### *The Secondary Transition*

This stage is characterized by the major wave of growth and differentiation toward the three lineages of pancreatic, acinar, ductal, and islet cells (Pictet et al., 1972). In this stage, the epithelium grows outward and forms MPCs around the periphery

of the epithelium. This branching happens for several days till the population of the MPCs dwindles completely. After E14.5, these progenitors differentiate into endocrine, acinar, and duct cells that are committed to exocrine fate (Zhou et al., 2007). Complete differentiation of β- and α-cells takes place from the epithelial tissue between E13–15. During the secondary transition, several transcription factors and signaling molecules have been identified as pancreatic markers and define pancreatic lineages, as summarized below.

### Neurogenin 3 (Ngn3)

Ngn3 is a basic helix-loop-helix (bHLH) transcription factor, expressed at secondary transition from E9.5–16.5. Ngn3 plays an important role as a master regulator and activator of gene transcription in endocrine progenitor cells during pancreatic development. Moreover, Ngn3 expression stimulates NeuroD1, Pax4, Nkx2.2, and Rfx6 that are involved in additional differentiation and subtype specification of different endocrine hormones produced by pancreas. Ngn3 is important for differentiation of endocrine cells, and its expression considered as signs of an endocrine pancreas (Dassaye et al., 2016). Loss of Ngn3 expression leads to a failure in the development of all pancreatic islet cells including α-, β-, δ-, ε-, and PP-cells, and thus, the hormones produced by them (Heller et al., 2005). Therefore, targeted disruption of Ngn3 in humans causes the failure of developed islet growth, neonatal diabetes mellitus, and early death (Schwitzgebel, 2014). High level of Ngn3 expression is essential to direct progenitor pancreatic cells into the endocrine cell fate and to initiate endocrine differentiation. While, low Ngn3 expression improves both acinar and duct cells development (Wang et al., 2010).

### Neurogenic Differentiation Factor 1 (NeuroD1)

NeuroD1 is a bHLH transcription factor, expressed at E9.5 in a subset of pancreatic epithelial cells and later expressed in α-, β-, and δ-cells. It is essential for β-cell maturation and maintenance of glucose response (Dassaye et al., 2016). Targeted disruption of Neurod1 in mice leads to reduction of insulin-producing cells and a decrease in glucagon-producing cells resulting in diabetes and early death of newborn mice (Naya et al., 1997). Homozygous mutation of NeuroD1 in humans results in the development of neonatal diabetes along with cerebellar hypoplasia (Rubio-Cabezas et al., 2011).

### V-Maf Musculoaponeurotic Fibrosarcoma Oncogene Family Protein A and B (MafA) and (MafB)

The Maf family of proteins is a subgroup of the basic region-leucine zipper (bZIP) transcription factors. The Maf protein family has two main groups according to

their molecular size as large or small Maf proteins (Motohashi et al., 2002). The large Maf proteins include MafA and MafB. Both are regulators for tissue-specific gene expression and cell differentiation in pancreas and other organs (Hang & Stein, 2011). MafA is activated by transcription factors such as Pdx1 and Nkx2.2 (Raum et al., 2006). MafA plays a critical role in activation genes significant for β-cell role, such as insulin (Aramata et al., 2005). Interestingly, the islet-enriched transcription factors, such as Pdx1 and Ngn3 together with MafA, have the ability to reprogram adult pancreatic acinar cells to β-like cells in mice. MafA with Pdx1 induces the development of β-cell from Ngn3-positive endocrine precursors and also permits Pdx1 to produce β-cells from α-cells and adult acinar cells (Dassaye et al., 2016; Zhou et al., 2007). MafB plays important roles in a variety of cell development, which is essential for differentiation α- and β-cell (Artner et al., 2010). It is expressed in α-cells of adult pancreas and is important for their function. During pancreas development, MafB is generated in glucagon and insulin cells. MafB is expressed earlier than MafA, detected pancreatic epithelium around E10.5, and MafA production was detected at E13.5 only in insulin cells (Hang & Stein, 2011).

### Paired Box Genes 4 and 6 (Pax4) and (Pax6)

The paired box (Pax) gene family consists of nine developmental control genes (Walther et al., 1991). Pax4 was expressed in dorsal and ventral buds at E9.5 but the expression was limited to β-cells and not found in adult islets while the loss of Pax4 resulted in the loss of β-cells it also resulted in the increase of glucagon cells (Sosa-Pineda et al., 1997). Pax6 is expressed between E9.5–E10.5 throughout the ventral and dorsal pancreatic buds, but later its expression is restricted to endocrine cell lineage (Turque et al., 1994). Moreover, it has a main function in the formation and cell differentiation of endocrine pancreas, brain, and various organs. In addition, Pax6 developmental regulator is critical for adult mouse maintenance of glucose regulation and of the endocrine role (Hart et al., 2013). A homozygous deletion of the Pax6 in mice caused diabetes and a decreased number of islet cell types (Sander et al., 1997). The mutations in Pax6 in humans lead to neonatal diabetes (Solomon et al., 2009).

### Aristaless-Related Homeobox (Arx)

Arx encodes a transcription factor belonging to the Aristaless-related paired-class homeobox proteins (Bienvenu et al., 2002). Both transcription factors, Arx and Pax4, have roles in differential endocrine cell subtype specifications. Moreover, both Arx and Pax4 factors exhibit antagonistic functions in endocrine specification (Collombat et al., 2003). Arx mutant mice showed upregulated Pax4 mRNA expression, while Pax4 mutant mice showed high levels of Arx mRNA. Arx is confined to α- and PP-cells fates and represses the β-/δ-cell lineage, whereas Pax4 promotes the β-/δ-cell lineages (Collombat et al., 2005). Arx-deficient mice showed hypoglycemia,

symptoms of dehydration and weakness associated with early total lack of adult α-cells, and increase in β- and δ-cell types (Collombat et al., 2003). In addition, double deficiency of Arx/Pax4 showed severe hyperglycemia with the loss of early developed α- and β-cell total and a significant rise in δ-cells numbers. Both cases of deficiency of Arx or double deficiency of Arx/Pax4 resulted in death of the animal in two days after birth (Collombat et al., 2005).

**Homeobox Genes (Nkx 2.2) and (Nkx 6.1)**

Nkx2.2 and Nkx6.1 are the NK class of homeodomain transcription factors encoding genes 2.2 and 6.1. Nkx2.2 expressed at E9.5 in the dorsal pancreatic epithelium which plays a main role in differentiation of β-cell. Nkx2.2 is also involved in the late differentiation of β-, α-, and PP-cells formation. It has a regulated role in endocrine cell differentiation by interacting with other transcription factors (Sussel et al., 1998). Nkx6.1 expressed at E9.5 in both pancreatic buds, whereas it is limited to developing β-cells at E14. It plays an important role in pancreas development. Nkx6.1 is also required for β-cells differentiation (Dassaye et al., 2016).

**Regulatory Factor X 6 (Rfx6)**

Rfx6 is a member of the regulatory factor X family of winged-helix transcription factors. Rfx6 is important for islet cell differentiation and insulin secretion in mice and humans (Aftab et al., 2008). It is expressed in the definitive endoderm at E7.5, then co-expressed with Nkx2.2. and Ngn3. At E9.0, it is limited to the pancreatic buds and later is limited to all adult endocrine cells (Dassaye et al., 2016). In humans, the mutations in Rfx6 lead to Mitchell-Riley syndrome, an autosomal-recessive disease of neonatal diabetes (Concepcion et al., 2014). At embryonic stages, Rfx6 knockout mice failed to develop any type of islet cell and died shortly after birth (Smith et al., 2010).

## *The Final Transition*

During final transition, the pancreatic differentiation cells undergo further remodeling, including other processes such as replication and neogenesis leading to the formation of adult pancreas. In this stage, the expansion of the ductal, acinar, and islet cells occurs. The islet cells are not fully developed till E19 up to 2 weeks after birth (Pictet et al., 1972). After differentiation, the endocrine cells delaminate, migrate into adjacent exocrine tissues, and aggregate into clusters of cells to form mature islets (Dassaye et al., 2016). Mouse islets are composed of 75% β-cells and 20% α-cells (Brissova et al., 2005). Mouse islets cells are organized as a central core of β-cells, which are enclosed by α-, δ-, ε-, and PP-cells (Prado et al., 2004). However, in

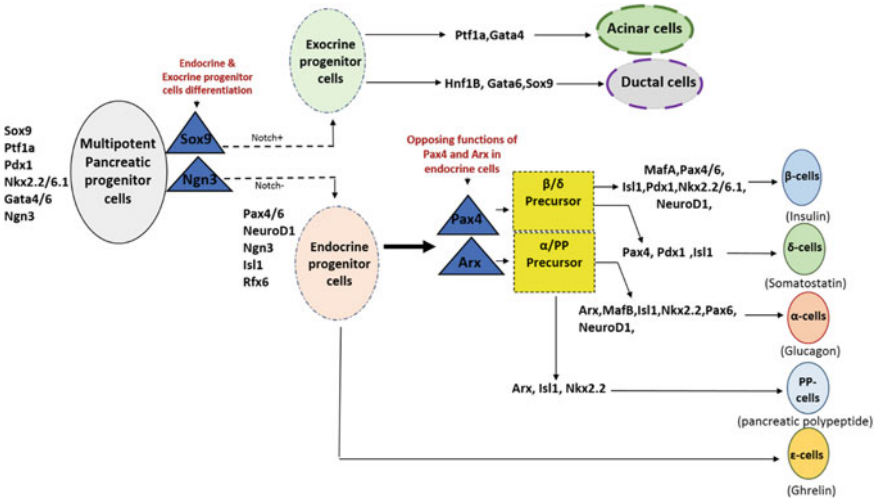


**Fig. 7.2** Transcription factors network regulating the pancreas development (Figure modified from Dassaye et al., (2016))

human, islets cells contain 50% of β-cells and 40% of α-cells (Brissova et al., 2005), and human β-cells are intermingled with α- and δ- cells through the islet (Cabrera et al., 2006). The schematic diagram in Fig. 7.2 summarizes some important keys of transcription factors during different transitions regulating pancreas development.

## Pancreatic Exocrine Cells

The exocrine pancreas part consists of two major cell types: acinar and duct cells. It accounts for at least 80% of pancreatic mass. Acini cells are organized into lobules with networks split into tubules. Each acinus is collected by pyramidal acinar cells (Frantz et al., 2012). The acinus (from the Latin term meaning "berry in a cluster") is specialized in synthesis, storage active enzymes, and inactive precursors (zymogens) and secret digestive enzymes into the duodenum (Williams, 2001). The acinar cells produce enzymes such as trypsinogen, chymotrypsinogen, carboxypeptidases, and nucleases. The acinar cell basal region has the nucleus and abundant rough endoplasmic reticulum for synthesis of digestive enzymes, whereas the apical region contains zymogen granules (storage for the enzymes). The digestive enzymes are secreted by the lumen of the acinar (Muallem et al., 1995). However, duct cells play essential roles for pancreatic exocrine activity by (**a**) producing both bicarbonate-rich fluid and mucins (that helps in neutralizing the stomach acid), in addition (**b**) forming extensive networks of tubules which transports enzymes for the digestive tract (Githens, 1988). The duct cells are ciliated, and polarized epithelial cells consist of cells that are cuboidal to pyramidal and have abundant mitochondria, that is

important for energy products and required for its ion transport functions (Benitez et al., 2012). Centroacinar cells located at the junction of the acinus and ductile, and this has a ductal cell characteristic. The duct cell as well as the centroacinar cells have carbonic anhydrase, and this is significant for their capability in secretion of bicarbonate (Steward et al., 2005).

## Pancreatic Endocrine Cells

The endocrine part represents only 2% of total volume of the pancreas. It is an aggregate of cells called the islets of Langerhans. The islet contains five main types of cells producing different hormones controlling and maintaining the homeostasis of glucose in the bloodstream include; **α-**, **β-**, **δ-**, **ε-**, and **PP-cells** (Frantz et al., 2012).

**α-cells** make up about 30–45% of the islet cells. Glucagon is released from α-cells in order to stimulate glycogenolysis in the liver when blood glucose levels are low (Freychet et al., 1988). Glucagon is a catabolic hormone that has an essential role in regulating glucose homeostasis in blood by stimulating hepatic glucose production (Röder et al., 2016). High glucose concentrations stimulate secretion of insulin from β-cells and inhibit secretion of glucagon, while low glucose concentrations (hypoglycemia) stimulate glucagon secretion (Brereton et al., 2015). Glucagon is released in response to hypoglycemic condition, prolonged fasting, exercise, and meals rich in protein. During long fasting hours, glucagon drives the liver and renal gluconeogenesis to increase endogenous blood glucose (Freychet et al., 1988).

**β-cells** produce the insulin hormone and make up 50–60% of each islet. The high level of blood glucose is a stimulator of insulin release (Komatsu et al., 2013). Insulin is an anabolic hormone; it is synthesized from β-cells as preproinsulin. A signal peptide from preproinsulin is cleaved into proinsulin in endoplasmic reticulum, where proinsulin is further cleaved by an endopeptidase identified as prohormone convertase to insulin and C-peptide. The resulting insulin is then stored in secretory granules by Golgi-apparatus waiting upon suitable stimuli for releasing from the cells. β-cell releases the insulin in response to various nutrients in the blood (Fu et al., 2013). Initially, for the release of insulin, glucose primarily enters into the β-cell through glucose transporter 2 (GLUT2) by means of a facilitated diffusion mechanism. GLUT2 is transmembrane located on the surface of the β-cells carrier proteins which permit passive transport of glucose into the cells (Marshall et al., 1993). It was expressed in β-cells, liver, and the expression was reduced in renal and absorptive cells of the intestinal. After entering β-cells, glucose is phosphorylated by glucokinase, which is a key enzyme that converts the glucose to glucose-6 phosphate (Efrat et al., 1994). The endpoint of glycolysis results in the surge of ATP/ADP ratio. This for a short period leads the cells into oxidative stress. A subsequent increase in the intracellular calcium levels due to oxidative stress results in the closure of ATP-sensitive $K^+$ channels (KATP). This results in the depolarization of membrane and dumping of insulin from the vesicles. Some products resulting from

these mechanisms acting as insulin secretion signals, such as NADPH, malonyl-CoA, and glutamate (Henquin, 2000).

**δ-cells** produce the hormone somatostatin and comprise only 5% of the islet cells (Cabrera et al., 2006). The δ–cells existing in the gastrointestinal tract, the central peripheral nervous system, and hypothalamus (Arimura et al., 1975). Somatostatin commonly known as somatotropin release-inhibiting factor or growth hormone inhibiting hormone (Brazeau et al., 1973). It is an inhibitor hormone released from the pituitary gland as well as an inhibitor of insulin and glucagon secretion (Youos, 2011).

**PP-cells** produce and secrete pancreatic polypeptide hormone (PP). These pancreatic cells are known as F-cells, which form the lowest amount of the total islet cells <5% (Cabrera et al., 2006). PP is a polypeptide hormone of 36 amino acids belonging to the peptide YY (PYY) and neuropeptide Y (NPY) family peptide (Kimmel et al., 1975). PP-hormone regulates the exocrine and endocrine production function of the pancreas. It is released rapidly into the circulation after food ingestion, cholinergic stimulation, and hypoglycemia. However, the glucose presence inhibits PP-hormone production. The physiological function of PP-hormone has not been established, but known to play a role in acid secretion and gallbladder relaxation, it effected upon digestive secretion and motility have been described (Frantz et al., 2012).

**ε-cells** comprise only <1% of the islet cells. These pancreatic cells produce the hormone ghrelin which is known as a hunger hormone (Pradhan et al., 2013). Ghrelin is a peptide containing 28 amino acids with n-octanoylation at serine 3 (Kojima et al., 1999). Mainly, it is produced in the stomach, increases secretion of growth hormones from the pituitary gland by growth hormone secretagogue receptor (GSH-R), and stimulates food intake and energy balance. Ghrelin has an essential role in secretory functions and development of pancreas. In addition, the majority of studies indicate that it has a functional in the glucose regulation that occurs by modulating insulin release (Pradhan et al., 2013). It increases blood glucose levels by suppressing insulin releasing from β-cells. Also, it has a role in β-cells growth and proliferation and prevents β-cell apoptosis (Sakata et al., 2019). Ghrelin signaling plays an important regulator role in obesity, insulin resistance, and diabetes. Interestingly, it has many regulatory physiological functions, most of these functions seem to be different from its effect on stimulating food intake, including cardiac functions, gastric motility stimulating, development of cancer, immunity, and inflammation system (Pradhan et al., 2013).

## β-cell Regeneration

Studying biological development of β-cell regeneration is essential for developing new treatments for diabetes. In both animals and humans, β-cell mass expansion slows significantly in adulthood due to very low rates of β-cell replication and neogenesis process (Teta et al., 2007). However, the regeneration of β-cells occurs during

different physiological and pathophysiological conditions such as pregnancy, obesity, and partial pancreatectomy.

## *β-cells Regeneration During Pregnancy*

Pancreatic β-cells regeneration occurs during pregnancy in humans and other animals. The maternal pancreas in pregnancy adapts to increased insulin resistance and metabolic demand by upregulating the proliferation β-cell mass which eventually returns to normal levels after delivery. This happens by decreasing β-cell proliferation and size and increasing its death (Sorenson & Brelje, 1997). Changes that affect β-cell mass increase in pregnancy time can lead to unregulated glucose homeostasis and gestational diabetes (Zhang et al., 2010). The proliferation of β-cells during pregnancy in rodents and humans is induced by placental lactogen (PL) and prolactin (PRL) hormones (Nielsen et al., 1999). It has proved this by experiment in which a short infusion of prolactin is enough to decrease menin (gene name multiple endocrine neoplasia type 1 MEN1) expression, which acts as a tumor suppressor blocked β-cell replication (Karnik et al., 2007). In pregnant rodents, β-cell proliferation is improved (two to five-fold) at gestational days 13–15 returning to normal levels at day 18–19 of delivery. Proliferation and hypertrophy of pre-existing β-cells are two of key developments of cells involved in this increase in β-cell mass (Ernst et al., 2011). In human pregnancy, there was an increase in the relative capacity of maternal islets and hyperplasia of β-cells (Van Assche et al., 1978). This the adaptive development in β-cell numbers is achieved by β-cell neogenesis rather than duplication of β-cells in existing islets (Butler et al., 2010). But, the clear proof of the proliferation process in these islet cells remains inconclusive and needs to be further investigated.

## *β-cells Regeneration During Obesity*

Another physiological condition of β-cells regeneration is the insulin resistance or obesity, where β-cell mass can multiply by several fold in obese mice. Using stained pancreatic sections from obese mice and humans, it has been detected as insulin producing cells that express Ki67 (a marker associated with cell proliferation). The regeneration β-cells ability is much higher in mice than in humans; however, the basic process is not completely clear (Butler et al., 2003).

### *β-cells Regeneration After Partial Pancreatectomy*

After pancreatectomy procedure, islet cells regeneration occurred in response to this injury. In rats, eight weeks after a 90% pancreatectomy, there was a regeneration to 27% and 45% of pancreatic weight and β-cell mass of sham-operated pancreas, respectively, (Bonner-Weir et al., 1993). The examination of potential of β-cell regeneration showed that in adult pigs 6 weeks after 60% pancreatectomy, there was a 19% increase in β-cell mass. Likewise, an 80% pancreatectomy caused a 56% increase in β-cell mass. Moreover, there was no improvement of insulin secretion or β-cell mass in the pancreas remnant in adult dogs after 80% pancreatectomy (Löhr et al., 1989). In humans, a 50% pancreatectomy does not prompt increased β-cell mass or regeneration. However, it causes impairments in secretion of insulin, and increases diabetes risk. Unlike humans, in mice diabetes does not develop spontaneously without changes in specific diet regimen, genetic predisposition, or chemical interventions. Therefore, differences between humans and rodents in β-cell turnover must be studied when estimating new treatment choices that aim to restore β-cell mass in diabetics (Menge et al., 2008).

## Pancreatic Diseases

Pancreatic disorders are divided into two categories depending on which part was affected, whether it is pancreatic exocrine or endocrine. The most common disorders that affect the exocrine pancreas are pancreatic cancer and pancreatitis. Pancreatic ductal adenocarcinoma is the most common type of pancreatic cancer. Diabetes and rare pancreatic neuroendocrine tumors affect the endocrine islets of Langerhans (Zhou & Melton, 2018). Pancreatitis is an inflammation of the pancreas that happens due to injury produced by enzymes action in pancreatic tissue. These enzymes are activated normally once they exit the pancreas; however, blockage due to infections or gallbladder stones can cause accumulation of these enzymes and activation within the pancreas. Pancreatitis can be acute or chronic. The most common causes of pancreatitis are alcohol consumption, cystic fibrosis, and hypercalcemia (Banks et al., 2010). Pancreatic ductal adenocarcinoma is poor differentiation of ductal/glandular structures which is believed to develop from progressive changes in the tissue. The prognosis of this disease is very poor with a low survival rate. Although the success of treatment is limited, there are many types of treatment. However, detection happens at the late stage of disease. Therefore, most of the current research is focused on early detection methods of pancreatic cancer (Castellanos et al., 2011).

### *Diabetes Mellitus*

Diabetes is the most common health challenges facing the modern world that creates a striking impact on health, society, and economy (Zhou & Melton, 2018). According to the latest data published in the International Diabetes Federation (IDF) Atlas, 9th edition illustrates that 463 million people worldwide are currently living with diabetes. DM is a metabolic disorder caused by an increase in blood sugar levels resulting from insulin resistance or a decrease in insulin production by the β-cells of pancreas or both. This disease leads to kidney failure, heart diseases, stroke, neuropathy, and retinopathy (Pagliuca & Melton, 2013). Diabetes is divided into two groups: type 1 and type 2 diabetes. Type 1 diabetes (T1D) produced by T cell-mediated autoimmune destruction of insulin-producing β-cells in the pancreatic causes insulin deficiency that leads to hyperglycemia (Ashcroft & Rorsman, 2012). The main susceptibility genes associated with diabetes are genes which regulate the human leukocyte antigen immune system (Singal & Blajchman, 1973). Type 2 diabetes (T2D) is impaired insulin production and insulin resistance, which is most often associated with different conditions such as age, obesity, and genetic factors. The most prevalent affecting approximately 85% of diabetic patients. Patients can be treated through diet, good nutrition, and exercise during the early stages of the disease (Ashcroft & Rorsman, 2012).

## Alternative Sources of Pancreatic β-cells

The main treatment for patients with type 1 diabetes is based on daily injection of exogenous insulin and combined with blood glucose monitoring. Although traditional insulin treatment supports blood glucose control levels, it is ineffective in the long term. Another alternative treatment is replacement of β-cells by transplantation of pancreas or pancreatic islets. However, it is currently challenging because there are many challenges facing these methods, including the shortage of human donor tissue, ethical conflict, and rejection of the organ by the immune system, use of immunosuppressive drugs, and other complications following these methods. On the other hand, type 2 diabetes is regulated by small-molecule drugs (such as phenformin, metformin) to stimulate the function of β-cell, to promote secretion sensitivity of insulin. In general, for patient type 1 diabetes (T1D) and advanced patient type 2 diabetes (T2D), it is difficult to control blood glucose perfectly by insulin therapy. Therefore, most of the researchers are trying to develop and find new strategies to generate pancreatic β-cells by focusing on the stem cell research, which received much consideration in this view and showed promising possibility as an alternate source of β-cells and other cells. Due to the regeneration and differentiation potential of stem cells, they are best candidates for diabetes treatment (Guney & Gannon, 2009; Peng et al., 2018). Although, there was difficulty in producing and developing the adult derived pancreatic β-cells in vitro, and there are many studies that have

used different stem cells models in order to obtain successful positive differentiation of β-cell in vitro. In order to provide diverse sources of β-cells for transplantation in diabetes patients, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and adult pancreatic stem cells and other cell sources. It will be mentioned in some of these sources in this chapter.

## *Differentiation of Embryonic Stem Cells (ESCs) into Pancreatic β-cells*

ESCs are pluripotent stem cells obtained from the undifferentiated inner cell mass of a blastocyst during embryonic development, and they have the capacity to differentiate into all cell types, including germ cells (Bradley et al., 1984). Over the past decade, various protocols have been described with the aim to mimic normal pancreatic development to produce pancreatic β-cells (insulin-secreting cells) in vitro. Some of these protocols were cultured mouse/human ESCs and iPSCs either in two-dimensional culture 2D methods, while other groups have used three-dimensional culture 3D methods. This is accomplished through the inclusion of several signaling molecules in the culture media that simulates those growth factors secreted from adjacent tissues such as sonic hedgehog, retinoic acid, and FGFs. Briefly, the ESCs differentiation development includes definitive endoderm formation and induction expression of Neurod1 and Ngn3, which they are critical in pancreatic growth, such as and finally the development of β-cell lineage by the induction of insulin and Nkx6.1 (Tse et al., 2015).

### Random Differentiation of Stem Cells into Pancreatic β-cells

The first positive protocols to promote differentiation of insulin-producing cells from ESCs used agents such as dimethyl sulfoxide (DMSO), nicotinamide, and antibiotic selection for isolating insulin-positive clones. The insulin-producing cells were able to restore hypoglycaemia in mice with diabetes; however, out of 784 clones, only 8 clones of geneticin-resistant cells were identified (Soria et al., 2000). Although this experiment provided evidence in principle that ESCs can produce insulin, the low success rate represented limitations to its clinical application. A parallel study confirmed that human ESCs (H9 cell line) were able to differentiate spontaneously into insulin-positive cells after cultured as embryoid bodies in suspension. These cells expressed transcription factors which are necessary for pancreas development as Pdx1, Ngn3, β-cell markers insulin, glucokinase, and Glut2 (Assady et al., 2001). These studies showed clear evidence that ESCs differentiate into endocrine cells spontaneously; however, the generated cell populations were very low, and they had a very low insulin content. This leads to insufficient differentiation development. Another attempt to improve the efficiency and yield of insulin-producing cells by

using signaling molecules to direct the ESCs toward differentiation in vitro (by means of a five-step protocol) (Lumelsky et al., 2001). This protocol depends on selected nestin-positive cells, which is an intermediate filament protein initially in neural stem cells (NSCs) of embryo and adult brains. It was used as a marker for stem/progenitor cell populations in different tissues (Lendahl et al., 1990). Nestin-positive cells were selected by plated embryoid bodies in medium containing insulin, selenium, transferrin, and fibronectin (ITSFn), expanded in medium containing FGF2, N2, and B27 supplements, and further differentiated by withdrawal of FGF2 in the presence nicotinamide. Even though the cells secreted insulin in response to glucose, these cells were also unsuccessful to normalize levels of blood glucose when subcutaneous transplantation into diabetic mice (Lumelsky et al., 2001). Several other studies found similar results by using mouse or human ES cell lines with a slight change in growth factors supplementation and found that there was an increase (30-fold) in insulin content when differentiated cells cultured in suspension (Baharvand et al., 2006; Kania et al., 2003). Other modifications applied according to the original protocol of Lumelsky et al. (2001) by replacing the B27 supplements with a phosphatidylinositol-3 kinase (PI3K) inhibitor at the last stage of differentiation. As a result, there was a 30-fold increase in insulin content. These cells prolonged survival, but they also failed to normalize the blood glucose level in transplanted animals. Another finding established that when using exendin-4 (its analog glucagon-like peptide 1 GLP1) or glucose-dependent insulinotropic polypeptide to differentiation culture. Cells significantly increased Pdx1 expression, insulin content and insulin secretion, resulting in reversal of hyperglycemia in diabetic mice (Lester et al., 2004).

### Direct Differentiation into Pancreatic β-cells

The production of β-cells directly occurred after differentiated cells, by a procedure named direct reprogramming, by passing the pluripotent condition (Takahashi et al., 2016). Generally, one should follow the normal endodermal pathway to produce β-cells from ESCs, due to the limitation of previous protocols in establishing a definitive endoderm progenitor population. This may be due to a lack of information regarding factors that stimulate the formation of the endoderm. Therefore, some studies used different culture conditions in order to mimic the properties of molecular signals which are known to initiate and/or control the development of pancreas from the endoderm *in vivo* (Zhou & Melton, 2008). The first differentiation of hESCs into definitive endoderm which was confirmed by expression of endodermal markers Sox17 and GATA4 (D'Amour et al., 2005). Using a viral system to express certain genes, such as Ngn3, Pdx1, and MafA, have successfully promoted insulin-producing cells from acinar cells in adult mice (Zhou & Melton, 2008), when these cells were transplanted in diabetic animals it resulted in normalized blood glucose in these animals. Retinoic acid is another molecule required for pancreas development in mouse embryos. It significantly induces Pdx1 expression by ESCs progenies. Some protocols used it alone or mixtures with other factors such as activin-A, sodium butyrate, FGF2, and nicotinamide (McKiernan

et al., 2007). Different studies improved earlier protocols of differentiation and generation of pancreatic β-cells by using novel small molecules. A seven-stage protocol was described to produce β-like cells from hESCs which maintained the expression of Pdx1, Nkx6.1, and NeuroD1, while also expressing MafA, which is a key β-cell maturation transcription factor. These cells were generated in serum-free conditions and addition of small molecules improved pancreatic specification and improved the generation of Pdx1 and Nkx6.1. These molecules include growth differentiation factor 8 (GDF8), GSK3β inhibitor (Inhibition of Glycogen Synthase Kinase 3β), FGF7, vitamin C, Retinoic acid, TPB ((Trifluoromethyl)phenyl)-2,4-pentadienoylamino)benzolactam), LDN (signaling inhibitor), and Sonic hedgehog agonist-1 (SANT-1) (Rezania et al., 2014). Moreover, a group of small molecules were used, such as R428, ALK5 inhibitor II, and N-acetyl cysteine (N-Cys), which might promote pancreatic β-cells differentiation with MafA expression at stage (S) 7 cells named (S7). The S7 cells are able to ameliorate hyperglycemia when transplanted into diabetic mice. However, these cells performed similarly, yet not identically to human β-cells. Additional studies carried out a modified protocol that showed improved glucose-responsive cells, which displayed properties similar to β-cells and an enrichment of Nkx6.1 and C-peptide expression. They tested several combinations of compounds and growth factors to generate stem cell-derived β (SC-β). They also found that the SC-β cells were able to generate insulin-secreting cells and with important expression markers of β-cells maturation such as MafA. Moreover, the SC-β cells have the ability to maintain euglycemia after transplanting into recipient mice (Pagliuca et al., 2014).

## *Induced Pluripotent Stem Cells (iPSCs)*

The iPSCs are resulting from reprogramming of human skin cells and other cells (Takahashi et al., 2016). Generation pluripotent stem cells (iPSCs) generated from somatic cells by the transduction of the main four stem cell transcription factors, namely Oct3/4, Sox2, Klf4c, and c-Myc (Takahashi & Yamanaka, 2006). The iPSCs have ESCs properties, and they can proliferate and self-renew in vitro and differentiate into different germ layers. These cells are similar to ESCs in morphological, surface antigens, expression genes and epigenetic status of pluripotent cell-specific genes (Takahashi et al., 2007). However, some differences have been discovered between hESCs and hiPSCs regarding the profiles of gene expression, genetic stability, and epigenetic modifications, for instance, DNA methylation profiles, stability of genomic imprinting, potential epigenetic modifications, and ability to model disease. The iPSCs were not identical to ESCs due to their leftover memory of their somatic origin. This memory may affect their protection, but there is no confirmed evidence if this memory can be fatal in cellular therapies (Shahjalal et al., 2018). Furthermore, the recent development in induced iPSCs has led to the avoidance of ethical debate of using hESCs. Studies have been established that induced insulin-secreting cells from iPSCs generated from fibroblasts, and these cells have

properties similar to differentiated ESCs (Tateishi et al., 2008). Pancreatic β-cells generated from iPSCs are a useful technique for analyzing pathological type 1 and 2 diabetes if the cells are generated from iPSCs established from diabetic patients (Pagliuca et al., 2014). The original protocol for iPSCs generation is used the retroviral or lentiviral-mediated expression of Oct3/4, Sox2, c-Myc, and Klf4. This was not an appropriate method to generate iPSCs that can be used in therapeutic applications due to the risks caused during insertion mutations and use the c-Myc oncogene, which produced tumorigenesis in chimeric mice obtained from these cells (Takahashi et al., 2007). Some of the studies have established iPSCs generation using non-integrating methods of gene delivery with potentially reduced risks such as plasmid transfection, episomal plasmid vectors, the PiggyBac transposon, and adenoviral transduction (Mayhew & Wells, 2010).

## *Differentiation of Mesenchymal Stem Cells (MSCs) into Pancreatic β-cells*

Mesenchymal stromal cells (MSCs) are heterogeneous population of stromal stem cells, which have capacities to differentiate into different cell types of all germ layers producing osteoblasts, adipocytes, myoblasts, and endocrine cells (Pittenger et al., 1999). MSCs isolated and cultured from several tissues such as bone marrow, skin, fat, umbilical cord blood, and placenta (Hass et al., 2011). Furthermore, MSCs are able to adhere to plastic in culture, easy to maintain under standard culture conditions, expansion in vitro and not only have the highest capacity to proliferate but also they can retain their pluripotent features even after different number of passages (Ueyama et al., 2012). Moreover, MSCs secrete factors, such as chemokines, cytokines, which improve the tissue microenvironment under injury conditions (Tögel et al., 2007). MSCs regulated the adaptive and innate immune systems by inhibiting both cells T- and B-activation and proliferation, inhibiting the dendritic cells differentiation, inhibiting proliferation and cytotoxicity of natural killer (NK) cells (Wang et al., 2018). They have low antigenicity, thereby reducing the toxicity (Solis et al., 2019). All these properties make MSCs a good alternative source for producing differentiated cells for therapy compared to other stem cell types. Interestingly, MSCs have potential to differentiate into insulin-producing cells (IPCs) in vitro by adopting specific methods (Chen et al., 2004). The microenvironment has an essential functional in the stem cells differentiation and survival, where it was found that conditioned medium prepared from redeveloped pancreatic tissue after partial pancreatectomy might promote rat bone marrow (BM)-MSCs to differentiate into IPCs (Choi et al., 2005). It was used rat BM-MSCs to differentiate islet cells, and transplantation of these cells reduces glucose level in non-obese (NOD) diabetic rats (Wu et al., 2007). It was also confirmed that human MSCs produced from the Wharton's jelly of the umbilical cord which has the potential to differentiate into islet cell clusters. These islet-like clusters can produce insulin in vitro and in vivo (Chao et al.,

**Table 7.1** Summary of some advantages and disadvantages of different stem cell types used in diabetes treatment (Lilly et al., 2016; Shahjalal et al., 2018)

| Cells type | Advantages | Disadvantages |
|---|---|---|
| ESCs | • Highly pluripotent differentiation capacity<br>• Unlimited self-renewal capacity | • Limited source with ethical issues<br>• Risk of tumor development after transplantation<br>• Immune rejection problems |
| iPSCs | • iPSCs have ESCs properties<br>• Easily obtainable as a source of stem cells without ethical issues | • Risk of tumor formation after transplantation<br>• Mutagenic potential in reprogramming procedures |
| MSCs | • Easy to isolate and expand without ethical issues<br>• High immunomodulatory properties | • Replicative lifespan is limited<br>• Contamination risk during differentiation and manufacturing in large amounts |

**ESCs**: embryonic stem cells; **iPSCs**: induced pluripotent stem cells; **MSCs**: mesenchymal stem cells

2008). MSCs obtained from Wharton's jelly can be used for xenotransplantation, as they do not show any stimulation of immune rejection responses (Weiss et al., 2008). Two methods used in vitro to promote MSC differentiation into IPCs. The first method is using genetic engineering to modulate gene expression via introducing key transcriptional regulatory Pdx-1 and Beta2 (Wu et al., 2007). The second method is using culture medium with specific soluble inducers or small-molecule compounds for inducing and promoting β-cell differentiation (Parnaud et al., 2008). An extract injured pancreatic tissue of rat was used for MSCs differentiation into IPCs using traditional two-step induction protocol. In stage 1 of protocol, BM-MSCs were induced with EGF, B27, and bFGF. In stage 2, serum-free high-glucose culture medium with activin-A, betacellulin, hepatocyte growth factor, nicotinamide, and other cytokines was used. Nicotinamide promotes fetal pancreatic cell differentiation, increases the amount of β-cell, and helps to synthesize insulin, while activin-A and betacellulin induce the differentiation of MSCs into β-cell. It indicated that the derived IPCs were effective in vivo and able to reverse hyperglycemia in diabetic rats (Xie et al., 2013). Some of the advantages and disadvantages of stem cells used for diabetes treatment are summarized in Table 7.1

## *Differentiation of Adult Pancreatic Stem/Progenitor Cells into Pancreatic β-cells*

Adult pancreatic stem cells are considered as potential sources of β-cells as they have the characteristics of stem cells including clonogenicity, multi-potency, and self-renewal. It has been suggested that all pancreatic exocrine cells, pancreatic ducts cells, and the islets of Langerhans are potential sources of a pancreatic stem/progenitor

cells. The cells of pancreas, such as ductal cells and acinar cells, share the same embryological origin with β-cells, which can be differentiated and re-programmed for insulin production (Kim & Lee, 2016; Pan et al., 2019). It is observed that almost all ductal cells express Pdx-1, which is important for pancreas development, especially in islet neogenesis of β-cells (Heimberg et al., 2000). Therefore, it was speculated that the ductal cells are the main source of new islet cells in the formation of new islet cells (Liao et al., 2007). Insulin-producing human islet-like clusters may be developed from human ductal tissue (Bonner-Weir et al., 2000). Using ductal tissue from a mouse or human reported the potential of generating islet-like clusters with identical or replicated protocols (Gao et al., 2003). The ductal cells probably switch to a less differentiated stage of expressing Pdx-1, as result the ductal cells acted as progenitor cells in the mature pancreas. Several investigations have tried to identify stem/progenitor cells in the pancreas (Bonner-Weir et al., 2004). Nestin is an important neural stem cell marker. Stem cells expressing nestin were isolated from human and rat pancreatic islets, and these cells can be cultured in vitro for a long period and have the ability to form insulin producing cells (Zulewski et al., 2001). The spherical neural stem cell clusters which were previously isolated from islet and ductal cells of pancreas show a precursor phenotype of both pancreatic and neural lineage. The progenitor cells have the ability to induce different populations of neurons and glial cells and also differentiate into pancreatic endocrine α-, β-, and δ-cells. Moreover, generated β-like cells derived from these progenitors demonstrate glucose-dependent $Ca2^{+}$ responsiveness and insulin secretion (Seaberg et al., 2004). The adult pancreatic stem cells successfully differentiate into islet-like cells. The human pancreatic ductal cells proliferate and differentiate into IPCs in vitro using combinations of growth factors, extracellular matrix proteins, and transcription factors (Corritore et al., 2016). In addition, ductal epithelial cells are considered as a source of pancreatic progenitors that can be generated in adult pancreas in diabetic mice after the partial pancreatectomy (Bhartiya, 2016). These results indicate that the existence of stem/progenitor cells in the pancreas might be a hopeful source for in vitro generation of islet cells, which can be useful in diabetes treatment. However, the specific marker identification is required to improve isolation populations of these cells (Pan et al., 2019).

## Generation of Pancreatic β-cells Using the Three-Dimensional 3D Cell Culture Method

### *Why Use 3-D Cell Culture Systems?*

3D cell culture is a more suitable technique used to study stem cell differentiation. 3D cultures can be produced in three different methods, such as cultures in matrigel, cultures on scaffolds, or suspension cultures on non-adherent plates and using floating culture. Different methods and materials are used in 3D cultures to

simulate and mimic the environment growth in vivo, and this allows cells to grow and migrate in 3-D full space (Wang et al., 2019). The 3D cultures provide different physiological features for testing the delivery and toxicity of drug. Also, it offers many advantages including the interaction between cells-cells and cells-extracellular matrix. The cellular heterogeneity of spheroid generated from 3D culture can closely mimic the morphological cells in vivo as well as their functions such as proliferation and induce the differentiation of cells which is helpful to its function, gene expression, etc., (Mehta et al., 2012). Interestingly, the morphology and polarity of the cells are maintained in 3D cultures, and they can be returned to cells before cultured in 2D (Kapałczyńska et al., 2018). All these features made 3D culture a powerful tool to increase stem cell differentiation or to support the cells reaching to the last stage of differentiation. However, 3D culture involves high cost and consumes more time (Wang et al., 2019). In contrast, the 2D monolayer cultures have low-cost culture maintenance and are simple to use. The 2D culture provides unlimited access to the components of the user medium as oxygen, nutrients, and molecules. However, the 2D cultures have many limitations, such as cells growing in 2D cannot mimic a growth environment in vivo. It cannot provide cell-cell and cell-extracellular environment interactions (Kapałczyńska et al., 2018)

## *Generation of Pancreatic β-cells Using the 3D Cell Culture Method*

The 3D cell cultures offer several advantages and have increased the interest of many research groups to generate pancreatic progenitors and insulin-producing cells from ESCs/iPSCs in vitro. The iPSCs derived from type 1 diabetes (T1D) patients were used to generate glucose-responsive and IPCs by using 3D culture methods. T1D iPSCs were originally shown resistant to differentiation, but the demethylation treatment effects showed a major improvement in IPCs yield. These cells release insulin in response to high-glucose stimulation in vitro. Moreover, these cells showed similar shape, size, and number of their granules that originated in cadaveric β-cells. The IPCs were transplanted into immunodeficient mice with streptozotocin (STZ)-induced diabetes, and hyperglycemia was gradually reduced. It can be considered that T1D iPSCs-derived β-cells are a suitable candidate for diabetes treatment (Manzar et al., 2017). However, it still needs a more efficient culture system that can be useful for future research and clinical applications. Organoid is once such a promising alternative, which is a 3D cellular cluster in vitro consisting of a group of primary cells, ESCs, or iPSCs. It has the capacity to regenerate to new cell types, self-regulate, and show functions similar to the original tissue in vivo (Fatehullah et al., 2016). The islet-like organoids clusters obtained from human pluripotent stem cells (hPSC) were capable of glucose-responsive insulin secretion and have therapeutic effects which could be used as alternative sources for diabetes treatment. It was demonstrated that the pancreatic endocrine cells (ECs) differentiated from hESCs allowed

the formation of cell clusters with 3D structures (100–150 μm in diameter). Moreover, the hESC-derived clustered endocrine cells secreted insulin and other pancreatic endocrine hormones. These EC clusters (ECCs) improved the secretion of insulin response to glucose (Kim et al., 2016). The generation of islet organoids would be valuable for research in diabetes pathophysiology, treatment, and screening of drugs (Wang et al., 2017). Generally, in the absence of capillary vessels in the 3D islet-like structure, the physiological oxygen circulation is insufficient. Both oxygen circulation and extracellular matrix (ECM) are essential for the reconstruction of the pancreatic β-like cells in differentiation. Therefore, components of the ECM such as laminin, collagen, and fibronectin membranes were used to control the tension of oxygen (Thakur et al., 2020). The development of islet organoids from hESCs in biomimetic 3D scaffolds. Matrigel and collagen type I used to form biocompatible scaffold, a porous, for supporting pancreatic islet differentiation. The porous plays a main function by supplying the cells with suitable energy, nutrients, and oxygen. Organoid biomimetic scaffolds could mimic the in vivo environments and also support (ECM)–cell and cell–cell interactions, which is an important regulator of cellular developments that help several functions. The organoids resulted from this study consist of α-, β-, δ-, and PP-cells. Remarkably, the generation of insulin-secreting cells did not co-express glucagon, somatostatin, or pancreatic polypeptide. The expression of Pdx1, MafA, Ngn3, and Glut2 was noticed in cell clusters in 3D culture. The cells grown in the scaffolds showed an increase in insulin expression compared to those grown in 2D cultures (Wang et al., 2017). A mixture of polycaprolactone (PCL) and polyvinyl alcohol-based (PVA) scaffold has been used to differentiate hPSCs into pancreatic lineage cells. The PCL/PVA has an important function in maintenance of the microenvironment, metabolic activation, and the expression of transcription factors needed for pancreatic cell differentiation. A study was carried out to investigate hiPSCs differentiation ability to insulin-secreting cells in which 3D culture was compared with 2D culture. The expressions of Insulin, Pdx1, Glut2, and Ngn3 in PCL/PVA scaffold were significantly higher than those expressed in 2D cultures. These results showed that the improved differentiation of IPCs from hiPSCs might be a result of PCL/PVA nanofibrous scaffolds used (Abazari et al., 2018). Additionally, Amikagel system permitted the coaggregation of hESC-pancreatic progenitor cells and endothelial cells by which pancreatic organoids, were closer to natural islet physiology, are formed in vitro. Amikagel encouraged spontaneous pancreatic progenitor spheroids differentiation into β-like cells, showing C-peptide protein expression and the capacity of glucose stimulation in vitro (Huang et al., 2020). Decellularization is the procedure used in biomedical engineering, in which ECM is separated from its native cells of a tissue or organ and retaining the real structure, biochemical, and biomechanical signals for producing a natural 3D scaffold, which might allow the integration of features like vasculature. This process could be accomplished using different methods including physical, chemical, and biological with each method having both advantages and disadvantages (Gilpin & Yang, 2017). The decellularized rat pancreatic ECM (dpECM) can induce self-assembly of human islet organoids during induced iPSCs differentiation. The iPSC-derived islet organoids of dpECM were secreted main hormones including

**Table 7.2** Summary of some attempts that used different protocols to differentiate stem cells into pancreatic β-cells in vitro and in vivo

| Cell line | Culture technique | GSIS | References |
|---|---|---|---|
| Mouse ESCs | Bacterial Petri dish culture; suspension culture (8–10 days) and the results of embryoid bodies were plated onto plastic cell culture dishes | Yes | Soria et al. (2000) |
| Mouse ESCs | 2D cell culture | Yes | Lumelsky et al. (2001) |
| Human ESCs | 2D cell culture | No | D'Amour et al. (2005) |
| Human MSCs | 2D cell culture | Yes | Chao et al. (2008) |
| Human ESCs<br>Human iPSCs | 2D cell culture (stages 1–4); 3D suspension culture (stages 5–7) | Yes | Rezania et al. (2014) |
| Human ESCs<br>Human iPSCs | Suspension-based culture system | Yes | Pagliuca et al. (2014) |
| Human ESCs | Matrigel-coated 4-well plates and suspension culture | Yes | Kim et al. (2016) |
| Human ESCs | 3D collagen scaffolds method | Yes | Wang et al. (2017) |
| T1D human iPSCs line | Matrigel /3D cell culture | Yes | Manzar et al., (2017) |
| Human iPSCs | 2D cell culture (18 days); 3D suspension culture (10 days) | Yes | Bi et al. (2020) |

**ESCs**: embryonic stem cells; **iPSCs**: induced pluripotent stem cells; **MSCs**: mesenchymal stem cells; **GSIS**: Glucose-stimulated insulin secretion; **T1D**: Type 1 diabetes

glucagon and insulin. These organoids contained α-, β-, δ-, and PP-cells. The exposure of iPSCs to the dpECM at differentiation stage showed higher expression of Pdx1, MafA, and Nkx6.1 (Bi et al., 2020). Table 7.2 summarizes some methods used to differentiate different types of stem cells into β-cells.

## Transdifferentiation of Pancreatic Cells

Transdifferentiation is a term that refers to changes in the cellular phenotype, such as conversion of differentiated cell type to another (Tosh et al., 2002). It is a process of phenotypic plasticity in a mature cell. Phenotypic change occurs in chronically damaged tissues and in tissue regeneration (Shen et al., 2000). It is considered as the most attractive method of developing β-cell sources which can be used for cell therapy. This procedure is based on cell reprogramming including neogenesis and regeneration of β-cell from progenitor cells (Kim et al., 2019). The transdifferentiation in the pancreas is acinar to ductal metaplasia (ADM) and is the process where acinar cells differentiate into duct cells, which plays a role in regeneration injured pancreas. Moreover, under certain microenvironment conditions, the acinar cells can

differentiate into hepatocyte-like cells and adipocytes (Lardon et al., 2004). The overexpression of polymorphisms of T cell factor 7-like 2 induced ductal epithelial cell proliferation and differentiation into islet-like clusters (Shu et al., 2012). The AR42J cell line derived from a pancreatic tumor, which has features of pancreatic acinar cells can transdifferentiate; by reprogramming these cells toward β-cells phenotype using of Pdx1, Ngn3, and MafA, induction of endocrine markers was observed (Akinci et al., 2012).

## Clinical Trials of Stem Cell Therapy for Diabetes

There is a growing global interest in stem cells research and the possibility to use it for treating various diseases such as diabetes. Stem cells have great therapeutic potential in this field. They have the potential for self-renewing, repairing damaged tissues cells, immunomodulatory properties, and their ability to provide an unlimited source of insulin-producing β-cells (Pathak et al., 2019; Peng et al., 2018). There have been several attempts of human clinical research studies using different types of stem cells in diabetic treatment, and some of these applications are summarized in this chapter. The first human clinical trial used autologous nonmyeloablative hematopoietic stem cells transplantation (AHST) to treat recent type 1 diabetic (T1D) patients. Also, it was evaluated the safety and metabolic effects of immunosuppression therapy. The results showed that most newly T1D patients accomplished different times of insulin independence and treatment-related toxicity was acceptable, no mortality reported. Moreover, with AHST, β-cell function was improved promisingly. However, this study needs further follow-up to confirm the time of insulin independence, randomness sample, and a control group (Voltarelli et al., 2007). A clinical study was carried out to estimate the effects of AHST in clinical and molecular processes in 9 recent T1D patients. The results showed that AHST increased the islet cell function due to removal of the islet specific autoreactive T cells; the difference in T1D patient reactions to AHST could be referred to these different transcriptional actions in the peripheral blood mononuclear cell (Zhang et al., 2012). Although less clinical trials have been performed in developing stem cell therapy for T2D, some encouraging results have been reported. It was studied the combination of intrapancreatic autologous stem cell (ASC) infusion with hyperbaric oxygen treatment (HBOT) in 25 T2D patients. In the follow-up period, hemoglobin A1c (glycosylated hemoglobin) levels were decreased, the insulin dose requirements reduced and increased C-peptide levels. These results suggest that ASC infusion and HBOT have positive therapeutic effects for T2D patients by improving metabolic control and reducing insulin requirements (Estrada et al., 2008). However, this study requires randomized controlled samples to confirm it. Another study that evaluated the combination of autologous bone marrow stem cell transplantation (ABMSCT) and (HBOT) on 31 T2D patients. Significant reductions in the dose of oral hypoglycemic drugs and decreased exogenous insulin dose have been demonstrated in all patients who used this therapy, but the functional development of pancreatic β-cell may be transient (Wang et al., 2011).

The intra-arterial injection of stem cells derived from bone marrow to T2D patients showed positive results, which confirm the efficacy and safety of this treatment for diabetics (Bhansali et al., 2014). An example of current human clinical trial which used stem cells in diabetes treatment was a pilot study of the therapeutic possibility of educator stem cells treatment in T1D phase 1 for both genders (18 years and older) in Hackensack, US. The aim of this study was to achieve patient's apheresis and then have their own blood returned to them with the "educated" lymphocytes (ClinicalTrials.gov NCT02624804). It was found that the stem cell educator treatment can develop the clinical treatment of diabetes and other diseases by cord blood-derived multipotent stem cells (CB-SCs) immune education and immune balance induction without the ethical and safety issues associated with traditional stem cell methods (Cheng et al., 2016).

## The Challenges of Stem Cell Therapy

Stem cells therapy is a promising potential therapeutic method for treating diabetes. Nevertheless, the results of stem cell clinical trials for diabetes treatments need further improvements to make them readily available for treatments. There are many challenges and obstacles that remain to be resolved in adopting this technology. The major challenges include how to (**a**) generate more developed functional β-like cells *in vitro* from hPSCs; (**b**) improve the efficiency differentiation of IPCs from hPSCs; **(c)** protect transplanted IPCs from autoimmune system; (**d**) generate enough numbers of cell types that required for clinical transplantation trial; (**e**) establish overall of insulin independence; and (**f**) avoid the carcinogenic properties that stem cells form, and maintaining the function and integrity of their stem cell-like characteristics in their production development (Chen et al., 2020). One of the most challenging goals that must be faced and overcome when using stem cell therapy is the immune rejection of the host. ViaCyte Inc. has successfully developed an encapsulation system named "Encaptra," in which microencapsulated pancreatic progenitors derived from stem cells are implanted subcutaneously to T1D patients in a phase 1 and 2 trial to evaluate efficacy and safety (ClinicalTrials.gov NCT02239354). The encapsulation device can provide a physical barrier protecting transplanted cells from the immune system while allowing oxygen and nutrients to pass through the membrane. Also, this system allows protecting patients from the risk of stem cell-derived β-cell oncogenic transformation (Chen et al., 2020; Sneddon et al., 2018). Sernova a clinical company, is developing a treatment for T1D (ClinicalTrials.gov NCT01652911) using implantable therapy device named "Cell Pouch," containing a scaffold with chambers that allows islet cells to vascularize, mimicking an environment similar to a natural organ. This device is inserted under the skin for a month to allow integration of vascular with the surrounding tissues (Sneddon et al., 2018). Systems like these can be potential solutions for future researchers to develop protocols using stem cells.

## Summary

The rate of diabetic disease is frighteningly increased around the world. The absence or loss of insulin-producing β-cells causes diabetes. The traditional treatment of diabetes has many limitations and cannot mimic natural pancreatic insulin production. As an alternative treatment for diabetes, islet transplantation maintains glucose homeostasis, and it is limited due to the lack of islet donation and other complications. Therefore, it is significant to determine advanced approaches to gain functional β-cells. Stem cell therapy offers a powerful promising potential for treating diabetes. Several types of stem cells have been proven effective in treating diabetes with clear limitations such as (ESCs), (iPSCs), (MSCs), and adult pancreatic stem cells. In conclusion, further human stem cell clinical trials are needed to overcome the challenges associated with stem cell and to make stem cell therapy a viable option for treating diabetes in the future.

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