A Review on the Progress of Stem Cell Therapy as a Treatment for Diabetes Mellitus

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Declaration

It is hereby declared that

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2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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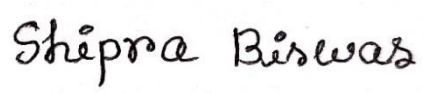

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Approval

The thesis titled “A review on the progress of stem cell therapy as a treatment for diabetes mellitus” submitted by Shipra Biswas (16346035) of Summer, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on January 2022.

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Ethics Statement

This is to certify that this project titled “Stem cell therapy on Diabetes Mellitus” is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, Brac University constitutes my own work under the supervision of Md. Tanvir Kabir, Senior Lecturer, Department of Pharmacy, Brac University and I have given appropriate credit where I have used language, ideas or writings of another. This project does not involve any human or animal trials. No animals were used or harmed in this project.

Abstract

The present scenario has shown that diabetes mellitus is a major health issue that affects people of nearly all ages. Stem cell therapy is one of the many medical treatment methods to prevent diabetes mellitus. A variety of stem cells are cultured to treat diabetes including embryonic stem cells, adult stem cells, induced pluripotent stem cells. Stem cells have been found from various sources which are embryos, blood, brain, bone marrow, muscle, liver, dental pulp, heart tissues, etc. Several transcription factors such as PDX-1, Ngn-3, Ptf-1a, MafA, Nkx6-1, etc. and key signaling pathways such as hedgehog, Notch, SOX9, Wnt, etc. contribute a lot to carry out stem cell treatment. Some errors of stem cell therapy have been found such as teratoma formation, risk of graft rejection that are being researched to overcome in the future. This review summarizes the progress of stem cell therapy as a treatment for diabetes mellitus.

Keywords: Diabetes mellitus; Stem cell therapy; Embryonic stem cell; Adult stem cell; Transcription factors

Dedication

Dedicated to the Chairperson of the Department of Pharmacy, Prof. Dr. Eva Rahman Kabir and my supervisor Md. Tanvir Kabir

Acknowledgements

I would like to proceed by thanking the Almighty who is the source of our strength and knowledge which have enabled me to complete this project work with the full diligence necessary to complete the Bachelor of Pharmacy (B.Pharm) program.

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List of Acronyms

DM	Diabetes mellitus
GDM	Gestational diabetes mellitus
HLA	Human leukocyte antigen
GLP-1	Glucagon-like peptide-1
DPP-4	Dipeptidyl peptidase-4 inhibitors
IPCs	Insulin-producing cells
ESCs	Embryonic stem cells
ASCs	Adult stem cells
iPSCs	Induced pluripotent stem cells
OCT3/4	Octamer-binding transcription factor 3/4
KLF4	Kruppel-like factor 4
MSCs	Mesenchymal stem cells
FPG	Fasting plasma glucose
OGTT	Oral glucose tolerance test
HGF	Hepatocyte growth factor
VEGF	Vascular endothelial growth factor
FBS	Fetal bovine serum
SCNT	Somatic cell nuclear transfer

Chapter 1

Background

The term “diabetes mellitus (DM)” indicates a multi-etiological metabolous disorder marked by long-standing hyperglycemia with abnormalities of the metabolism of fats, carbohydrates as well as proteins. It is a disorder that affects the body’s capacity to help keep blood sugar levels consistent and also suppresses the secretion of insulin on target tissues due to insensitivity or insufficient insulin (Alberti & Zimmet, 1998). When the efficiency of pancreatic beta cells decreases, they produce little or no insulin. Lack of adequate insulin leads to hyperglycemia that characterizes DM (Pipeleers & Ling, 1992). Throughout the last few decades, the number of DM patients worldwide has increased fourfold. According to the Global Burden of Disease (GBD) report in 2013, the ninth leading cause of death is all forms of diabetes mellitus (Zheng, Ley, & Hu, 2018). Worldwide population surveys have shown that DM incidence is increasing and we are very likely to reach epidemic levels (Latif, Jain, & Rahman, 2011).

Several approaches to diabetes management have been devised up to now such as insulin therapy, pancreas transplantation, etc. However, several inconveniences had been observed with these treatments. Thereafter, researchers looked at an alternative treatment method which is stem cell therapy. In this thesis paper, several pieces of research on stem cell treatment in diabetes management have been reviewed. The purpose of my work is to recognize which aspects of stem cell therapy are more effective than other treatment methods, the inconvenience of this therapy, and how to achieve the best outcome from this therapy by eliminating the errors.

Chapter 2

Global Burden of Diabetes Mellitus

Throughout the last few decades, the worldwide occurrence of impaired glucose tolerance and diabetes in adults has grown. Since 2000, IDF has monitored the national, regional and worldwide incidence of DM. In 2017, 451 million people worldwide between the ages of 18 and 99 were estimated to suffer from diabetes and 693 million are expected by 2045. Nearly half (49.7%) of people living with diabetes were estimated to have not been diagnosed. In addition, impaired glucose tolerance (IGT) was estimated at 374 million people. Nearly 21.3 million women were estimated to have hyperglycemia during pregnancy. About five million people worldwide between the ages of 20 and 99 died due to diabetes.

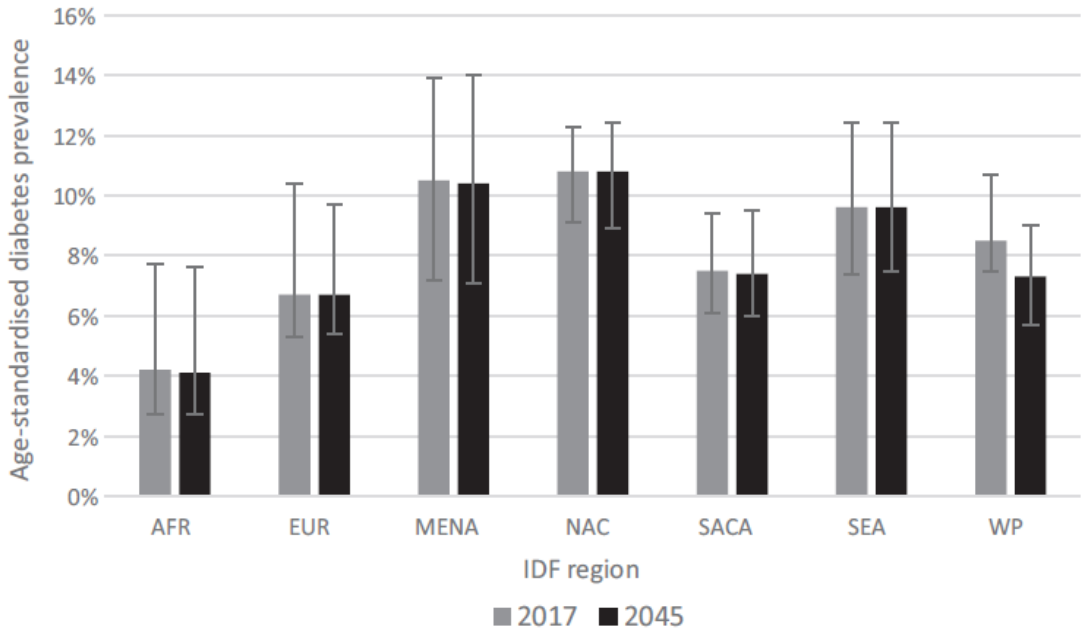


Figure 1: Age-standardised Prevalence of Diabetes Per IDF Region for 2017 And 2045 (Age Range:18-99), Adopted from, (Cho et al., 2018)

In several countries and regions, the rate of change in the incidence of diabetes has increased due to fast urban development and drastic shifts in the sedentary way of life. Diabetes

prevalence fluctuates based on the economic situation of the country. Diabetes prevalence peaked in low-income countries (8 percent) among 55-64 ages, peaked between 60 and 74 years (19 percent) in middle-income countries. Additionally, men are more likely to suffer from DM. There are around 12.3 million more men who are bearing this incurable disease than women who suffer from DM. Diabetes prevalence is predicted to increase to 9.9% in 2045 for both women and men (Cho et al., 2018). Approximately 1 in 11 people around the world suffer from diabetes mellitus, of which 90% people suffer from T2DM. The Asian continent is experiencing a dramatic rise in T2DM (Zheng et al., 2018). Adults in Africa face the lowest world-standardized prevalence of DM and the North American and Caribbean regions had the highest global diabetes prevalence (Ogurtsova et al., 2017). The prevalence of T1DM is mainly higher in Denmark, Norway, Finland, Iceland, Sweden and lower in Asia, Latin America as well as Africa. In South African Indians, a higher prevalence of T2DM is noted. In females, T1DM is less common compared to males. On the other hand, women are more prone to T2DM than men (Adeghate, Schattner, & Dunn, 2006). This unprecedented rise in diabetes places an immense burden on both healthcare providers and healthcare authorities (Latif et al., 2011).

Chapter 3

The scenario of Diabetes Mellitus in Bangladesh

In Bangladesh, approximately 10 million people suffer from diabetes (Mohiuddin, 2019). The recent condition of diabetes management in Bangladesh was assessed by DiabCare Bangladesh 2008. An analysis to explore existing diabetes management scenarios was conducted by 1952 patients from 01 March 2009 to 31 March 2009 in diabetes clinics, referral clinics and general hospitals. Data have been obtained from a total of 100 different centers in Bangladesh. This study includes both T1DM and T2DM sufferers. The DiabCare Bangladesh 2008 was intended to assess the present state of diabetes DM treatment, life expectancy, regulation, complications and psychosocial aspects of patients as cross-sectional, observational research to be correlated with previous studies to evaluate the results.

Results demonstrate that glycemic regulation deteriorates with 8.6±2.0% mean HbA1c with 23.1% of the patients have reached the American Diabetes Association (ADA) target of <7%. Even though 48 percent of the patients were on lipid-reducing drugs, 44.1 percent had HDL<1 mmol/L, 43.8 percent had triglycerides >2.2 mmol/L and 70.8 percent of patients had LDL levels >2.6 mmol/L. In 12.1 percent, 9.9 percent and 39.2 percent of patients, extreme late complications, macrovascular and microvascular problems have been identified respectively. According to the assessment of the standard of living, around half of diabetes patients in Bangladesh are of low living quality (Latif et al., 2011). The incidence of DM in rural Bangladeshi communities grew from 3.8 percent in 1999–2000 to 8.5 percent in 2004–2005 which indicates a day-by-day rise in diabetes prevalence (Jayawardena et al., 2012).

Chapter 4

Global Economic Burden of Diabetes Mellitus

In 2017, overall diabetes-related global health expenses were projected at US$ 727 billion for people aged 20 to 79 years. As the age class expands to eighteen to ninety-nine, this rate increases to US$ 850 billion. The worldwide spending on the healthcare sector will increase by 7 percent by 2045, reaching US$ 958 billion (18–99 years) and US$ 776 billion (20–79 years). With ID (international dollar) 445 billion, the North American along with Caribbean territory had the highest mean medical costs for diabetes (18-99 years), totaling 445 billion ID (international dollar). In addition, the lowest average healthcare expenses were found in Southeast Asia. The overall global diabetes expenditure was 52% in 2017. Additionally, a substantial part of overall global expenses was incurred for the West Pacific Region (ID 199bn) and the Europe Region (ID 224bn). Between the ages of 60 and 69 (US$ 127 billion), the utmost expenses were made for males with a 7 percent higher proportion than females. Women accounted for higher expenses than men in classes of 70–79 and 50–59 years.

In 2045, the medical spending of people under 50 years of age is projected to steady, however, the population over 70 years of age would rise by 37 percent (Cho et al., 2018). The average annual cost per patient was substantially linked to insulin use, female gender, presence of diabetes complications and a longer period of diabetes. The cost per annum for diabetes patients primarily comprised of direct expenses such as laboratory testing, hospital care and medicines. The cost of medications was the highest share (60.7 percent) of the total direct cost, led by hospitalization expenditures (27.7 percent). Moreover, for patients that did not need hospitalization, medicine was also the most expensive direct expense (83.5%). Medicinal costs were taken into consideration for 50.7 percent of the direct expenses for hospitalized patients, with hospitalization costs accounting for 39.9%. Patients who were hospitalized had an

estimated annual indirect expense of US$158.9, which was almost four times higher than patients who were not hospitalized (US$41.8) (Afroz et al., 2019).

Chapter 5

Types of Diabetes Mellitus

The National Diabetes Data Group (NDDG) categorized diabetes mellitus as insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus into two groups in 1979. Based on pharmacological therapy, diabetes mellitus was categorized in this way. Four types of diabetes mellitus were recognized by the ADA in 1997 based on pathogenesis. The following categories are included: type 1 diabetes mellitus, type 2 diabetes mellitus, other types of diabetes mellitus and gestational diabetes mellitus. This is the more well-known categorization of DM (Maraschin, 1997).

5.1 Type 1 Diabetes Mellitus

Insulin-dependent diabetes is a term used to describe type 1 diabetes mellitus (T1DM). Juvenile-onset diabetes is another name for it. This is because insulin, particularly during puberty or childhood, is important for treating type 1 diabetes mellitus. But it can occur in individuals of any age. In the case of T1DM, very little amount of insulin or no insulin is produced from pancreatic β cells due to the depletion of pancreatic β cells. T1DM occurs in 5% to 10% of patients. Such patients are often vulnerable to autoimmune disorders such as Addison's disease, pernicious anemia, Graves' disease, vitiligo, Hashimoto thyroiditis, myasthenia gravis, celiac sprue and autoimmune hepatitis (Diabetes, 2010).

5.2 Type 2 Diabetes Mellitus

Another form of DM is type 2 diabetes mellitus (T2DM). Adult-onset diabetes, or non-insulin-dependent diabetes, is another name for it. This is because insulin is not necessary for T2DM patients to survive and people over the age of 35 are more likely to have it. The body produces insufficient insulin or prevents the effects of insulin for maintaining blood glucose levels in the case of T2DM. T2DM is extensive among 90%-95% of diabetic patients. Most patients are

obese in this kind of diabetes and obesity contributes to insulin resistance. Women who have gestational diabetes mellitus are more likely to evolve T2DM. Patients who have trouble with dyslipidemia and hypertension are also at risk of T2DM (Diabetes, 2010).

5.3 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a condition identified first in the period of gestation in women (Diabetes, 2010). It is characterized as any level of glucose intolerance that starts or becomes apparent during pregnancy. GDM is complicated in about 7 percent of all births, with over 200,000 cases a year. The incidence may be between 1% and 14% of all pregnancies. Glucose tests should be carried out as soon as possible on women with clinical attributes associated with high-risk GDM (GDM history, significant obesity, strong family history and glycosuria) (Singh & Rastogi, 2008). It can be minimized with the help of diet. If it is not controlled through diet, then insulin therapy has to be taken (Coustan, 2013).

5.4 Other Forms of Diabetes Mellitus

Other forms of DM include hereditary problems in insulin action, genetic errors in the function of beta-cell, other genetic syndromes occasionally associated with DM, infections, medicinal products or chemically induced diabetes, rare types of immune-mediated diabetes, exocrine pancreatic diseases as well as endocrinopathy. More diabetes-related genetic disorders include Turner syndrome, Prader-Willi syndrome, Wolfram syndrome, Klinefelter syndrome, Down syndrome, Myotonic dystrophy, Huntington chorea, Friedreich ataxia, Porphyria and Laurence-Moon-Biedl syndrome. pancreatectomy, fibrocalculous pancreatopathy, cystic fibrosis, pancreatitis, hemochromatosis, and neoplasia are the diseases of the exocrine pancreas. Diazoxide, Dilantin, Vacor, Nicotinic acid, Pentamidine, etc are all medications that can induce diabetes mellitus (Diabetes, 2010).

Chapter 6

Etiology of Diabetes Mellitus

Multiple etiologies of occurring diabetes mellitus have been found. Environmental and genetic factors also keep a role in the progression of DM (Kommoju & Reddy, 2011). Medications and drug toxic agents, physical inactivity, obesity, locations and viral infections are environmental factors that keep contributing to diabetes development (Adeghate et al., 2006).

6.1 Genetics

HLA-DR4, HLA-DR3 or both are carried by 95 percent of type 1 diabetes patients. DR4 enhances the hazard of T1DM by 9 times and DR3 increases the risk by 7 times. But the risk is 14 times higher than normal when both DR3 and DR4 are present (Adeghate et al., 2006).

6.2 Dietary Factors

Cow's dairy protein, N-nitroso and nitrites compounds intake may increase the hazard of growing T1DM in the infant. Then, the occurrence of autoimmune diabetes has been shown to increase with the solvent-extracted protein derived from wheat gluten, gluten and the addition of wheat gliadin to the diet. Inadequate levels of activated vitamin D can also progress the chance of T1DM in children (Åkerblom, Vaarala, Hyöty, Ilonen, & Knip, 2002).

6.3 Age

The most significant factor that affects diabetes prevalence is age. The prevalence rises with age but in some countries, it may plateau or even fall in old age groups (75 and up). According to data from Nutrition Examination Survey and the third National Health in the United States, men aged 75 and up have the highest prevalence of diabetes at 21.1 percent. Women have a peak prevalence of 17.8 percent which occurs between the ages of 60 and 74 (Jain & Saraf, 2010).

6.4 Gender

According to some evidence, women suffer more from diabetes. A higher rise in men diagnosed with diabetes has led to a similar prevalence rate of men and women in some populations in recent years (Jain & Saraf, 2010).

6.5 Country of Residence

Since diabetes is related to age, countries that are with older populations have more diabetes incidence than those with younger populations in developing countries. The prevalence rates in Europe are between 3 to 10%, while the rate ranges of Asian Indian, Arab and Hispanic American communities from 14 to 20%. The Pima Indians in the United State, a Pacific Island and natives of Nauru with a prevalence of up to 50 percent have been shown to have higher rates of DM (Jain & Saraf, 2010).

6.6 Place of Residence

Within the same region, the DM prevalence in urban is significantly higher than in rural areas. Diabetes is also more prevalent in urban societies when migrant populations living in rural and urban settings in the same country are compared (Jain & Saraf, 2010).

6.7 Ethnicity

Several studies have shown that ethnicity has been linked to the prevalence of DM. According to the National Health and Nutrition Examination Survey (NHANES) 111 report, the progression of DM is 1.9 times higher in Mexican Americans, while 1.6 times higher in non-Hispanic blacks than non-Hispanic whites. A local study found diabetes in Asian men four times higher than in white men (Jain & Saraf, 2010).

6.8 Viral Infections

According to several studies, viral infections may keep contributing to the progression of T1DM in humans. An example of virus-induced diabetes in humans is congenital rubella. Additionally, more than 30 years ago, first studies on enterovirus infection with T1DM showed that the seasonal difference in diabetes incidence results from enterovirus infections (Åkerblom et al., 2002). Moreover, in 20–30 percent of newly diagnosed cases of T1DM, IgM antibodies against Coxsackie B4 have been identified (Adeghate et al., 2006).

6.9 Obesity

Diabetes and obesity have reached alarmingly high levels globally, with a minimum prevalence of 170 million and 300 million respectively. Obesity is a distinct risk factor for DM. The risk of DM has been doubled by obesity (Jain & Saraf, 2010).

6.10 Drugs

Some medications such as oral contraceptive steroids and corticosteroids that are responsible for glucose intolerance can cause T2DM (Adeghate et al., 2006).

6.11 Lifestyle

Some ethnic groups are especially prone to diabetes when moving from a rural to an urban lifestyle. Asian Indians, North American Indians, Polynesian Pacific Islanders and Australian Aborigines have all been found to have this trait (Jain & Saraf, 2010).

6.12 Diseases

Patients with autoimmune diseases as rheumatoid arthritis, Addison’s disease, autoimmune thyroid disease and pernicious anemia are more prone to T1DM (Adeghate et al., 2006).

Chapter 7

Symptoms of Diabetes Mellitus

Distinctive symptoms of diabetes mellitus such as blurred vision, thirst, polyphagia, polydipsia, polyuria and weight reduction may occur (Diabetes, 2010). Depression is another symptom of diabetes (Eker, 2018). GIT symptoms such as diarrhea, constipation, frequent abdominal pain, ulcer-like dyspepsia, bowel-related pain, fecal incontinence, dysmotility like dyspepsia and gastroesophageal reflex are also raised if the hyperglycemic state cannot be controlled (Bytzer et al., 2002). The symptoms are either not serious or may not be present (Diabetes, 2010). Mainly the signs and symptoms are not serious or missing in the case of type 2 diabetes (Yamamoto-Honda & Akanuma, 2002). In addition, higher levels of blood glucose, severe glycosuria as well as ketonuria are the common symptoms of children with diabetes (Alberti & Zimmet, 1998).

Chapter 8

Complications of Diabetes Mellitus

Patients face various complications due to the prevalence of diabetes mellitus (Diabetes, 2010). Long-term effects increase the chance of ulcers on the foot, amputation, Charcot joints, ESRD and autonomic disorder leading to sexual, genitourinary, gastrointestinal abnormality (Diabetes, 2010). People may also suffer from hypertension and a disorder of lipoprotein metabolism. Long-term injury, failure of multiple organs and malfunction are the common complications of DM. Organ failure is particularly related to the blood vessels, nerves, eyes and kidneys. In serious cases, uncontrolled ketone development or a non-ketotic hyperosmolar condition can occur. If effective therapies are not introduced, stupor, coma, and death will result (Diabetes, 2010).

8.1 Microvascular Complications

Microvascular complications include diabetic retinopathy which gradually causes partial or complete vision loss, focal blurring, and retinal or vitreous detachment that can be treated with or vitrectomy laser photocoagulation (Giorda, Manicardi, & Diago Cabezudo, 2011). Additionally, various types of neuropathy such as autonomic neuropathy, cranial neuropathy, symmetric polyneuropathy, mononeuropathy, radiculopathy which may contribute to renal failure and neuropathy can occur due to long term impacts of diabetes mellitus. These complications can cause severe morbidity (Giorda et al., 2011).

8.2 Macrovascular Complications

Macrovascular complications of diabetes mellitus are peripheral arterial disease, cardiovascular disease such as angina pectoris and MI and cerebrovascular disease includes transient ischemic attack as well as stroke. Cardiovascular disease can lead to percutaneous trans-luminal coronary angioplasty or CABG surgery, which can even lead to heart failure

(Giorda et al., 2011). According to the International Diabetes Federation (IDF), around 75-80 percent of people who suffer from diabetes die because of cardiovascular complications.

Complications of DM	**Number of tests (n)**	**Number of patients (N)**	**Proportions of patients (%)**
Macro-vascular complications	1860	185	9.9
Micro-vascular complications	1860	729	39.2
Cataract	1087	140	12.9
Cerebral stroke	1704	37	2.2
Myocardial infarction	1579	82	5.2
Serum creatinine > 2mg/dl	997	30	3.3
Proliferative diabetic retinopathy	1059	15	1.4
Non-Proliferative diabetic retinopathy	1080	77	7.1
Angina pectoris	173	115	6.6
Neuropathy symptoms	1158	367	31.7
Absent foot pulses	825	48	5.8
Absent ankle jerk	822	337	41.1
Leg amputation	842	10	1.2
Microalbuminuria	212	34	15.7

Table 1: Proportion of Diabetic Patients with Various Complications (Latif et al., 2011)

Chapter 9

Tests for Diabetes Mellitus

There are four diagnostic tests currently prescribed for diabetes. The following tests are used to diagnose diabetes mellitus: Random plasma glucose (RPG) test, fasting plasma glucose (FPG), oral glucose tolerance test (OGTT) and HbA1c tests (Yamamoto-Honda & Akanuma, 2002). HbA1c is widely used as an indicator of chronic hyperglycemia representing blood glucose levels over two and three months (Diabetes, 2010). Symptoms such as elevated thirst and urine production, severe weight loss, frequent infections, drowsiness and coma are usually the cause of the clinical diagnosis of diabetes (Alberti & Zimmet, 1998).

The criteria for diagnostic confirmation differ individually from the asymptomatic person and the symptomatic person (Diabetes, 2010). Asymptomatic patient with a plasma glucose value of about 7.0 mmol/L (126 mg/dl), 2-h post-load plasma glucose of approximately 11.1 mmol/L (200 mg/dl), HbA1c of about 6.5% (48 mmol/mol) or random plasma glucose at an allocation of 11.1 mmol/L (200 mg/dl) is known as diabetes (Yamamoto-Honda & Akanuma, 2002). For an asymptomatic individual that can be either a fasting plasma glucose test or oral glucose tolerance test, a minimum of one extra blood plasma glucose test is needed. When it is not confirmed by the test for diabetes mellitus, the clinician must re-test it. In these cases, the physician should consider additional variables such as age, family history, concomitant disorder and adiposity along with abnormal blood glucose value before starting treatment (Diabetes, 2010). The requirements for diagnosis in children are the same as in adults. For children with less severe symptoms, most are diagnosed with a fasting blood glucose test, blood glucose test, or oral glucose tolerance test (Alberti & Zimmet, 1998).

Chapter 10

Current Treatments for Diabetes Mellitus

Drugs are categorized into two classes which are applied to treat diabetes. One is insulin and another one is oral agents. These two types of drugs are administered to minimize the higher level of blood glucose and suppress difficulties regarding diabetes mellitus. Different patients are treated with different medications as not all patients respond to the same types of medicines. They are insulin secretagogues, biguanides, thiazolidinediones (TZDs), DPP-4 inhibitors, meglitinide derivatives, alpha-glucosidase inhibitors and GLP-1 agonist (Pankaj Modi, 2007). In the case of children with diabetes, immediate medical treatment should be initiated (Alberti & Zimmet, 1998).

10.1 Medications

10.1.1 Insulin Secretagogues

10.1.1.1 Sulfonylurea Insulin Secretagogues

Insulin secretagogues include sulfonylureas promote insulin production in the body from pancreatic beta cells by blocking the ATP-sensitive k+ channel of beta cells which contributes to cell depolarization and eventually exocytosis of insulin. Sulfonylureas are classified into two categories, sulfonylureas in the first and second generations. For the patient, sulfonylureas of the second generation are safer and more potent. The first generation of sulfonylureas comprises chlorpropamide, tolazamide, acetohexamide and tolbutamide. The second-generation sulfonylureas include glyburide micronized (Glynase), glimepiride (Amaryl), glyburide (Micronase, Diabeta) and glipizide (Glucotrol). The two most common side effects of these medications are hypoglycemia and weight gain. Hypoglycemia is more common, especially in first-generation sulfonylureas due to the long half-lives of these agents (Pankaj Modi, 2007).

10.1.1.2 Non-sulfonylurea Insulin Secretagogues

Non-sulfonylurea secretagogues include meglitinide derivatives such as repaglinide (Prandin) and nateglinide (Starlix) and benzoic acid derivatives. Meglitinide binds to K_{ATP} channels as well, although on sites other than conventional sulfonylureas. The secretion of insulin is incited by blocking the K+ channel in β cells after administration of meglitinides. The aftereffects of meglitinides are weight gain and hypoglycemia which are similar to sulfonylureas (Pankaj Modi, 2007).

10.1.2 Biguanides

Biguanides include metformin which promotes glucose uptake in the muscles and decreases the production of hepatic glucose. Metformin is beneficial for overweight people as it does not grow fat like sulfonylureas. The negative consequences of metformin are diarrhea, nausea, lactic acidosis and anorexia. These side effects are seen in 30% of diabetic patients (Pankaj Modi, 2007).

10.1.3 Insulin Sensitizers

Thiazolidinediones (TZDs) such as pioglitazone (marketed as Actos) and rosiglitazone (marketed as Avandia) are insulin sensitizers that improve the function of insulin in fats, muscles as well as other tissues. These agents increase the sensitivity to insulin through PPAR-g receptor activation. Obesity and hydropsy are correlated with the principal side effects of thiazolidinediones (Pankaj Modi, 2007).

10.1.4 α-Glucosidase Inhibitors

Miglitol and acarbose are included in the alpha-glucosidase inhibitors. These medications decrease hepatic glucose production and carbohydrates absorption rate in the small bowel. The initial consequence of which is the decrease in postprandial plasma glucose levels.

Oral antidiabetics		Mechanism of action	Side effects
Meglitinides	Nateglinide (Starlix)	Blocking the K+ channel in ß-cells increases insulin secretion in the first phase.	Weight gain and hypoglycemia
	Repaglinide (Prandin)		
Sulfonylureas	Glipizide	Increase insulin secretion in the first phase by resisting K+ in beta cells.	Hypoglycemia, weight gain as well as late hyperinsulinemia
	Glyburide		
	Tolbutamide		
	Tolazamide (Tolinase)		
	Glimepiride (Amaryl)		
	Acetoheximide (Dymelor)		
	Glipizide-gits (Glucotrol-XL)		
	Chlorpropamide (Diabinese)		
	Glyburide micronized (Glynase)		
Thiazolidinediones	Pioglitazone (Actos)	Activating PPAR-g receptors stimulates insulin sensitivity.	Weight gain and fluid retention
	Rosiglitazone (Avandia)		

α- glucosidase inhibitors	Miglitol (Glyset)	Delay absorption of glucose and lower production of hepatic glucose	Abdominal bloating and flatulence
	Acarbose (Precose)		
Biguanides	Metformin-XR (Glucophage-XR)	Reduce production of hepatic glucose, increase the elevation of muscle glucose.	Diarrhea, nausea, lactic acidosis and anorexia
	Metformin (Riomet, Glucophage)		

Table 2: Mechanisms of Action and Side Effects of Oral Agents (Pankaj Modi, 2007)

Alpha-glucosidase inhibitors are administered when a patient's blood glucose levels rise dramatically after eating (postprandial). In combination with other medications, alpha-glucosidase inhibitors are most effective. Gastrointestinal side effects such as abdominal bloating, flatulence, abdominal pain and diarrhea can be seen among 30% of diabetic patients after administering these medications. But, consuming less carbohydrate in the diet can mitigate these side effects (Pankaj Modi, 2007).

10.1.5 GLP-1 Receptor Agonist and DPP-4 Inhibitors

The chemical substance (hormone) GLP-1 is produced in the small gut and released into the bloodstream within minutes after a meal containing carbohydrates or fat. Insulin secretion and synthesis are promoted by GLP-1. In addition, GLP-1 eliminates the release of glucagon and postpones stomach emptying. Due to rapid degradation by DPP-4, It has only a 12-minute half-life. For this reason, DPP-4 inhibitors and GLP-1 receptor agonists for the treatment of T2DM were developed. Sitagliptin, linagliptin, and saxagliptin are the three DPP-4 inhibitors that have received FDA approval (Quianzon & Cheikh, 2012).

10.1.6 Combination Therapy

Diabetic patients are also treated with a combination of medicines. If the target is not achieved with monotherapy, combination therapy is given to obtain better results. The majority of drug classes, apart from sulfonylureas or non-sulfonylurea insulin secretagogues, can be given combined because of the same mechanism of action. Combination tablets are designed to reduce the pill burden. Insulin therapy should be started if oral combination therapy fails to meet glycemic targets (Pankaj Modi, 2007).

10.2 Insulin Therapy

Insulin is the primary therapy in treating T1DM that is administered by an insulin pump or injection. Long-acting insulin named Insulin glargine (Lantus) is very beneficial for the treatment of T1DM (Devendra, Liu, & Eisenbarth, 2004). Daily blood sugar levels need to be checked and daily injections are necessary during continuing insulin treatment. There are many types of insulin includes injectable insulin and inhaled insulin.

Class	Also known as	Elucidation	Onset	Duration	Generic names
Long-acting insulin	Background insulin, Basal insulin	After rapid-acting insulin has stopped functioning, this medication keeps blood sugar under control. It takes time for the body to absorb and has a prolonged effect.	One to four hours	Eighteen to twenty-four hours	Detemir, Glargine (U-100)
Rapid-acting insulin	Mealtime insulin	It is typically administered prior to a meal to target sugars consumed during the meal. It acts rapidly and isn't extremely long-lasting.	Five to thirty minutes	Three to five hours	Aspart, Lispro, Glulisine
Short-acting insulin	Mealtime insulin	It is generally administered prior to a meal for the sugars consumed during meals. It acts quickly and is short-lived.	One to five hours	Five to twelve hours	Regular insulin (U-100)
Intermediate-acting insulin	Background insulin, Basal insulin	This medicine is administered to control blood glucose levels when rapid-acting insulin does not work. It is absorbed slowly by the body and works for the long term.	Two to four hours	Ten to eighteen hours	Neutral protamine hadegorn
Inhaled insulin	Mealtime insulin	This is ultra-rapid-acting insulin. Its pharmacokinetics	Twelve to	Two hours and	Afrezza

		and pharmacodynamics are more rapid. It is taken before mealtime.	fifteen minutes	thirty minutes to three hours	

Table 3: Different Types of Insulin ("Insulin Types - Western New York Urology Associates, LLC," n.d.) (Klonoff, 2014)

The treatment for T1DM also includes the bio-artificial pancreas, islet cells and vascular device transplantation which are alternatives to insulin therapy (Silva, Norton De Matos, Brons, & Mateus, 2006).

10.3 Diet and Exercise

Diabetes can be controlled by committing to daily exercise and maintaining a healthier diet (Pankaj Modi, 2007). Aerobic exercises such as fast walking, cycling, running and swimming are very beneficial for diabetic patients as they improve vascular activity. Diabetic patients should include nuts, vegetables, fruits, unrefined cereals, olive oil, fish, yogurt and legumes in their diet as these foods help to prevent DM (Walker, O'Dea, Gomez, Girgis, & Colagiuri, 2010). Additionally, they should intake stevia as a source of sweetener instead of sugar. Stevia contains stevioside which causes insulinotropic effects in the pancreas. It boosts the secretion of insulin and thus lowers the level of blood glucose (Hossain, Islam, Islam, & Akhtar, 2017). Moreover, side effects of antidiabetic drugs can be minimized through a lower amount of carbohydrate intake in the diet and continuing regular exercise (Pankaj Modi, 2007).

10.4 Gene Therapy

One of the possible treatment options for healing type 1 diabetes mellitus is gene therapy. Gene therapy is a method used as a therapeutic strategy for curing disease in which genetic material is delivered or manipulated inside the cell (Mali, 2013). It effectively stops or slows the

progression of the disease by correcting faulty genes that cause disease. Gene therapy consists of three major intervention techniques which are inserting a new gene into the body, replacing dysfunctional genes with functional genes and inactivating disease-causing genes (Kaufmann, Büning, Galy, Schambach, & Grez, 2013; Mali, 2013). Germline gene therapy which targets reproductive cells and somatic gene therapy which targets somatic cells to prevent disease progression in future generations are the two most common forms of gene therapy (Chellappan et al., 2018; Kaufmann et al., 2013).

Chapter 11

Stem Cell Therapy

Stem cells are known as non-specialized cells found throughout the body that possess the capacity of making precise, indefinite copies of themselves by mitotic cell divisions. They can distinguish and produce specialized cells in the different body tissues. In most multicellular species SCs are present. They exist in both embryonic and adult cells that means these cells are present in all people from the beginning of human growth until the termination of life (Kalra & Tomar, 2014; Ota, 2008). They exist in a variety of organs and tissues such as blood, brain, bone marrow, skin, muscle, liver, dental pulp and heart tissues (Islam, 2018; Shi & Gronthos, 2003). These are essential for our nerves, bones, brain, skin, blood or other organs to develop, grow, maintain and repair. The totipotency of SCs is one of their most distinguishing characteristics. Adult stem cells have multipotency while very early embryonic stem cells demonstrate their totipotency (Kalra & Tomar, 2014). In cell therapy, stem cells can be employed as substitutes to replace injured cells or reproduce tissues (Kolios & Moodley, 2012). SCs exhibit considerable therapeutic potential for diabetes sufferers because of their immunomodulatory capabilities and their capacity to reproduce into insulin-producing cells (IPCs) (Zhang, Chen, Feng, & Cao, 2020).

11.1 Classification of Stem Cells

Stem cells come in a variety of forms that depend on their potency and originality. Based on their origin, they can be classified into four broad categories. They are embryonic stem cells (ESCs), adult stem cells (ASCs), tissue-resident stem cells and induced pluripotent stem cells (iPSCs). Additionally, all stem cells can be divided into five groups by their potency which are pluripotent SCs, totipotent or omnipotent SCs, multipotent SCs, unipotent SCs and oligopotent SCs (Kolios & Moodley, 2012; Smith, 2006).

11.1.1 Stem Cell Classification Based on Origin

11.1.1.1 Adult Stem Cells

Adult stem cells can be either totipotent or multipotent. They occur throughout the entire body following embryonic growth which replenishes dying cells and regenerates damaged tissues by cell division. Adult SCs are primarily responsible for restoring as well as maintaining the tissue in which they are located in a living body (Kalra & Tomar, 2014).

11.1.1.2 Embryonic Stem Cells

Embryonic stem cells are pluripotent, auto-replicating cells with the ability to live indefinitely. ESCs are formed from embryos in a growth phase before usually appearing in the uterus during implantation. They are developed into a variety of embryonic cells (Avasthi, Srivastava, Singh, & Srivastava, 2008). Usually, embryos that are 4 or 5 days old represent a hollow microscopic cluster of cells known as the blastocyst (Kalra & Tomar, 2014).

11.1.1.3 Tissue-resident Stem Cells

Some of the tissues and organs in the adult are relying heavily on tissue-resident stem cells which produce terminally differentiated tissue-specific cells to heal and renew after injury (Kolios & Moodley, 2012; Passier & Mummery, 2003). According to research, these cells form during ontogenesis and remain dormant until they migrate, proliferate and differentiate through local stimuli (Kolios & Moodley, 2012; Voog & Jones, 2010).

11.1.1.4 Induced Pluripotent Stem Cells

Pluripotent stem cells have been produced from adult stem cells by Takahashi and Yamanaka through somatic cells reprogramming process which is referred to as iPSCs. These cells have comparable features to ESCs (Takahashi & Yamanaka, 2006). In 2006, Takahashi and Yamanaka reported mouse iPSCs for the very first time through the transduction of mouse fibroblasts with four genes that encode transcription factors such as octamer-binding

transcription factor 3/4 (OCT3/4), SRY-related high-mobility group box protein-2 (SOX2), Kruppel-like factor 4 (KLF4) and the oncoprotein c-MYC. The production of human iPSCs from adult human dermal fibroblasts was reported by Yamanaka and colleagues from the same four factors: SOX2, OCT3/4, KLF4 and c-MYC, one year later, in 2007 (Kolios & Moodley, 2012; Takahashi et al., 2007).

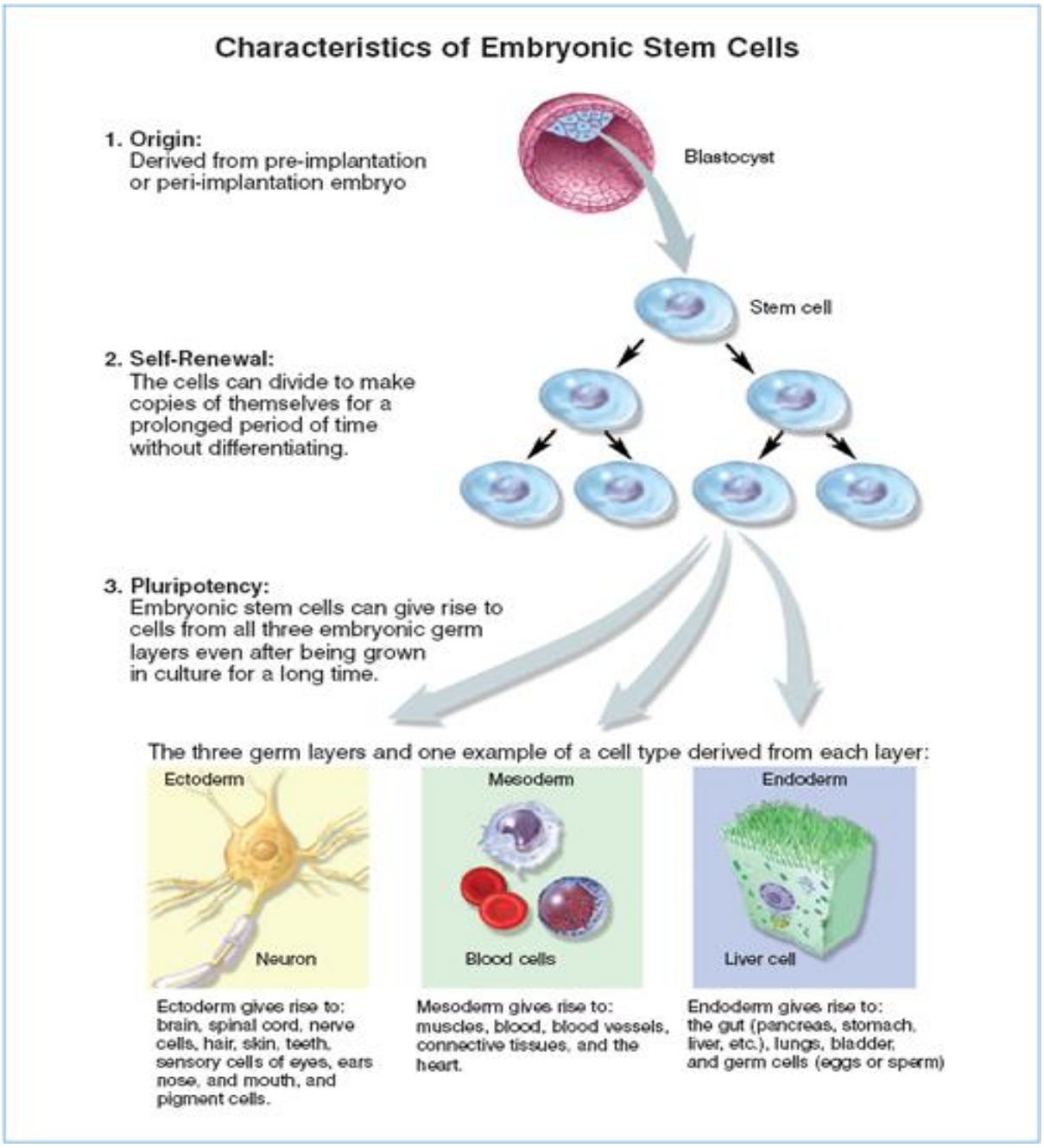


Figure 2: Characteristics of Embryonic Stem Cells, Adopted from, (Islam, 2018)

11.1.2 Stem Cell Classification Based on Potency

11.1.2.1 Totipotent Stem Cells

Totipotent or omnipotent SCs can split and differentiate into all kinds of cells throughout the organism (Ota, 2008). These types of cells can be found during the primary phases of growth (Kolios & Moodley, 2012). Totipotency possesses the greatest differentiation potential and allows cells to form embryonic and extra-embryonic tissues. As a result, the embryo and placenta are formed. The totipotent cells include a zygote formed after sperm fertilization of an oocyte (an immature egg cell) and the first two cellular divisions (Kolios & Moodley, 2012). The zygote forms a blastocyst through mitosis cell division which is a tiny ball of several cells (Kalra & Tomar, 2014). The blastocyst's interior cell lump transforms into pluripotent after around four days (Ota, 2008).

11.1.2.2 Pluripotent Stem Cells

Pluripotent stem cells can be differentiated into cells from three germ layers such as ectoderm, endoderm and mesoderm which serve as the foundation for the growth of all tissues and organs (de Miguel, Fuentes-Julián, & Alcaina, 2010). Extraembryonic structures such as the placenta are not formed from these types of cells (Ota, 2008). The blastocyst's interior cell cluster is the elementary state from which pluripotent stem cells, also familiar as ESCs, are generated (Evans & Kaufman, 1981; Kolios & Moodley, 2012).

11.1.2.3 Multipotent Stem Cells

The differentiation of multipotent stem cells is narrower than pluripotent stem cells (Ota, 2008). They are present in nearly all tissues and develop from a single germ layer into a close-related cells family (Kalra & Tomar, 2014; Kolios & Moodley, 2012; Ratajczak et al., 2012). For instance, hematopoietic stem cells can grow into blood cells. A hematopoietic stem cell can

differentiate into an oligopotent cell. Its capacity to differentiate is limited to its lineage cells (Ota, 2008). In addition, the most well-known multipotent cells are mesenchymal stem cells (MSCs). They may be formed from many kinds of tissues such as umbilical cord blood, adipose tissue, bone and Wharton's jelly, bone marrow as well as peripheral blood (Augello, Kurth, & de Bari, 2010). These cells can differentiate into tissue originating from the mesoderm such as adipose tissue, muscle, cartilage and bone (Augello et al., 2010; Friedenstein, Chailakhjan, & Lalykina, 1970; Prockop, 1997). The neuronal tissue that was formed from the ectoderm has recently been differentiated from MSCs (Barzilay, Melamed, & Offen, 2009; Kolios & Moodley, 2012a).

11.1.2.4 Oligopotent Stem Cells

Oligopotent stem cells can renew themselves and two or more lineages are formed in a particular tissue (Kolios & Moodley, 2012). For instance, leukocytes can be differentiated from myeloid stem cells, but not erythrocytes (Ota, 2008).

11.1.2.5 Unipotent Stem Cells

Unipotent stem cells are the least capable of differentiating (Ota, 2008). They are capable to self-renew and specializing themselves into only one type of cell, resulting in the formation of a single lineage, such as muscle stem cells, which exclusively create mature muscle cells (Beck & Blanpain, 2012; De Rooij & Grootegoed, 1998; Kolios & Moodley, 2012c; Overturf, Al-Dhalimy, Ou, Finegold, & Grompe, 1997). They can produce only their own kind of cells (Kalra & Tomar, 2014). For example, type 1 alveolar pneumocytes in the lungs can produce Type 2 pneumocytes (Kolios & Moodley, 2012).

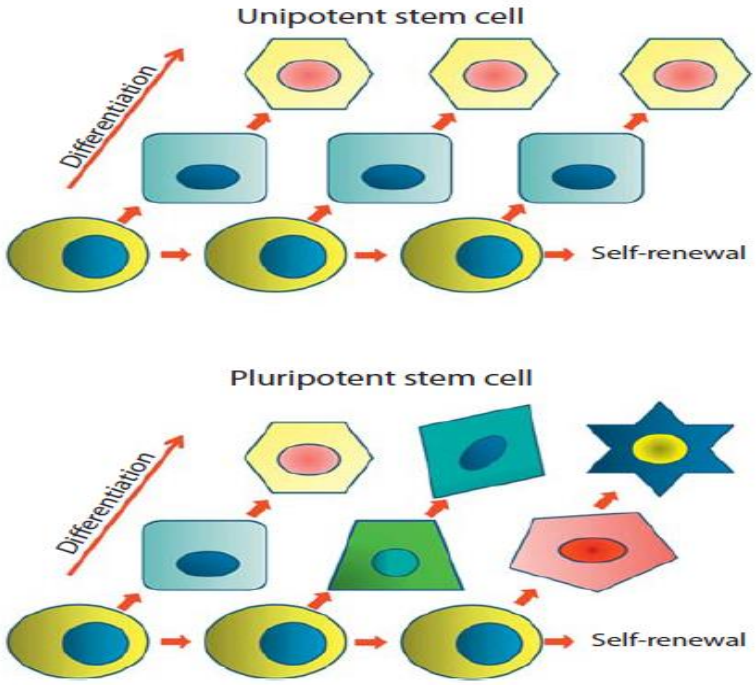


Figure 3: Differentiation of Unipotent And Pluripotent Stem Cell, Adopted from, (Kolios & Moodley, 2012)

11.2 Insulin to Stem Cell Therapy

Exogenous insulin injections are the traditional way of treating diabetes. However, the current insulin treatment cannot fully control glucose levels and results in long-term problems. Because, exogenous insulin is unable to sustain the optimum physiological glucose level and is frequently associated with hypoglycemia (Vija et al., 2009). Transplantation of islet cells and pancreas for beta-cell replacement therapy are two other alternative options for treatment (James Shapiro, 2008). Despite this large shift from conventional insulin to islet transplants, it confronts numerous obstacles including a scarcity of donors and lifelong immune suppression requirement to ensure long-term function and survival of human islet graft (Hansson & Madsen, 2010). As a result of this critical shortfall of pancreatic donors, researchers looked at an alternative supply of beta cells through stem cell transdifferentiation (Hogan, Pileggi, & Ricordi, 2008; Sarath & Rani, 2012).

11.3 Sources of Stem Cell

11.3.1 Embryonic Stem Cells (ESCs)

The first human embryonic stem cell (hESC) was isolated in 1995 by Thomson et al (Thomson et al., 1995). To promote the growth of hESCs, ESCs of Rhesus monkeys have been grown in culture medium. The source of hESCs is the core of the blastocyst (Lei et al., 2007). β cells can be differentiated from ESCs produced from mice and monkeys (Lester, Kuo, Andrews, Nauert, & Wolf, 2004; Soria et al., 2000). Schroeder et al. described an effective approach of differentiating ESCs into the pancreatic lineage (Schroeder, Rolletschek, Blyszczuk, Kania, & Wobus, 2006).

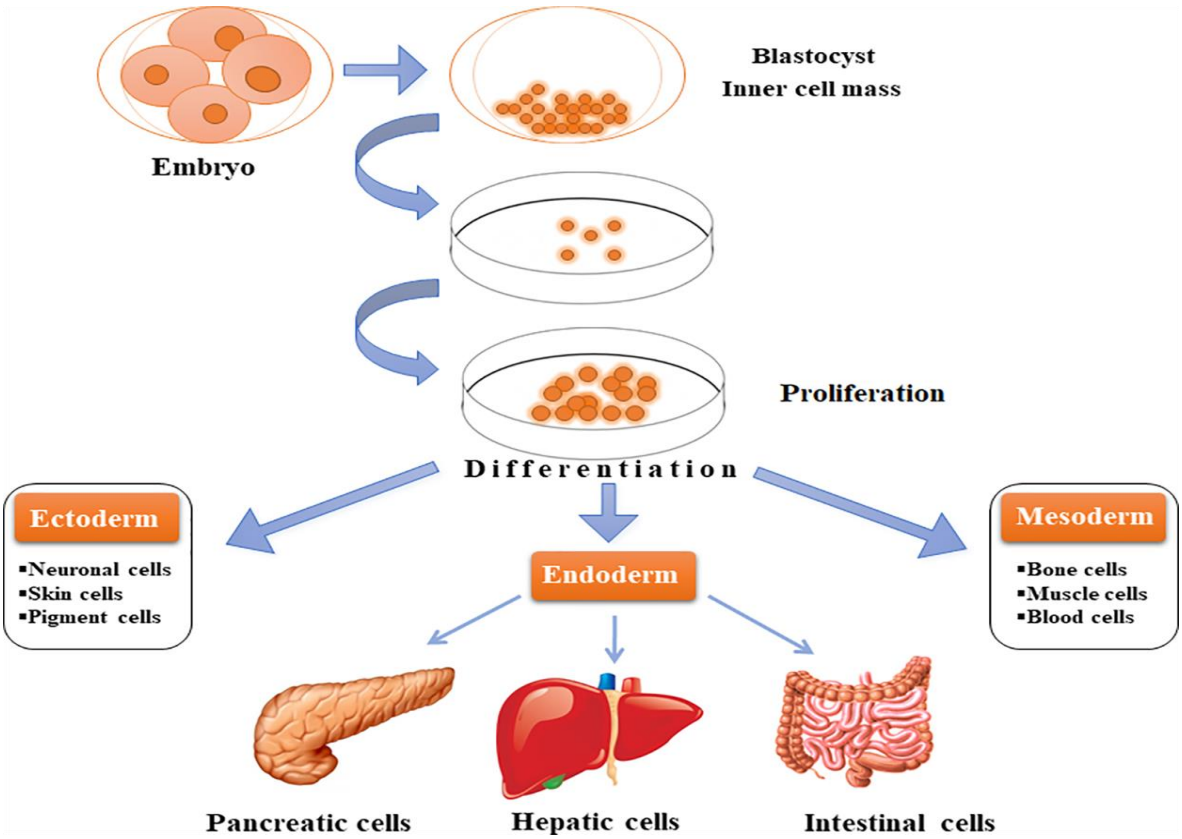


Figure 4: In Vitro Differentiation of Embryonic Stem Cells into A Variety of Cells, Adopted from, (Saleem et al., 2019)

Extracellular growth factors were used to induce the spontaneous creation of multilineage progenitor cells in this process, which were then differentiated into clusters of insulin-

producing beta cells. These cells released insulin when they were exposed to excessive quantities of sugar. For several months, diabetic mice and rats with hyperglycemia were given insulin-secreting cells derived from ESCs, which developed their condition (Saleem et al., 2019).

11.3.2 Umbilical Cord Blood Stem Cells

Stem cells exist in abundance in the umbilical cord blood. They are therefore enthusiastic about a wide range of areas, including regenerative medicine. In recent years, the clinical uses of such SCs have grown enormously. There is no danger to the donor, only a minor risk of growing graft-versus-host disease, no ethical concerns and it is readily available (Zhao & Mazzone, 2010). Two limitations for clinical applications of umbilical cell-based treatments are SCs dosing and compatibility with the human leukocyte antigen (HLA) system. DM can be treated with autologous SCs from cord blood using a new banking technique that does not require HLA matching. A patient's immune cells can also be trained to reduce autoimmunity by using SCs from cord blood. The lack of SCs in cord blood can constitute a significant obstacle to their utilization. The aforementioned difficulty can nevertheless be remedied by in vitro multiplication to grow an appropriate amount of cells while maintaining SCs characteristics (Saleem et al., 2019; Zhao & Mazzone, 2010). Blood stem cells and mesenchymal stromal cells (MSCs) can be found in abundance in umbilical cords which have been used as prospective origins of insulin-producing cells and immune modulators in T1DM (Aguayo-Mazzucato & Bonner-Weir, 2010).

11.3.3 Hepatic and Intestinal Stem Cells

A similar cell population appeared in the embryonic endoderm that develops the ventral pancreas, liver and intestine. The identical population of SCs may produce epithelial cells in the liver and pancreas. As a result, these cells are receiving increased attention from researchers. In response to high glucose concentrations during in vitro researches, the oval SCs

from the liver can be differentiated into β cells. Beta-cell transcription factors such as Nkx2.1, Pax6, insulin promoter factor-1 (Pdx-1), Nkx6.1 and Pax4 are clearly expressed in these differentiated cells (Nakajima-Nagata et al., 2004).

A further study found that Pdx-1 is expressed through the engineering of human fetal hepatic progenitor cells. These cells are transplanted to the diabetic patient to prevent hyperglycemia after insulin-producing cells are differentiated from these cells (Zalzman et al., 2003). Adult liver progenitor cells overexpress Pdx-1 when they are exposed to glucose, which leads to insulin production (Finegood, Scaglia, & Bonner-Weir, 1995). Researchers have recently used lentiviral vectors to successfully reprogram hepatic stem white blood cells into pancreatic endocrine type precursor cells while continually expressing Pdx1-VP16 along with Pdx-1. After being introduced to a diabetic patient, functional pancreatic β cells are converted from these cells and the hyperglycemic condition is reversed in the diabetic patient (Tang et al., 2006). These findings demonstrated that intestinal or hepatic SCs could be differentiated into pancreatic β cells under controlled conditions, indicating that they could be adequate sources of insulin production (Saleem et al., 2019).

11.3.4 Pancreatic Stem Cells

The pancreas ducts are a major source of β progenitor cells, as evidenced by various researches. The creation of new islet cells is aided by the continual differentiation of these ductal progenitor cells (Finegood et al., 1995). In periductal areas, islet cells can develop particularly after partial pancreatectomy in diabetic patients (Bonner-Weir, Baxter, Schuppin, & Smith, 1993). Rodent pancreatic ducts can produce islet-like clusters. When glucose is stimulated, these clusters exhibit proteins and islet genes, then insulin generation and secretion are occurred (Bonner-Weir et al., 2000). β cells are developed from multipotent precursor cells found both in ductal cell clusters and pancreatic islets in adults (Seaberg et al., 2004). The ectopic expression of a critical factor known as neurogenin 3 (NGN-3), keeps a crucial contribution in pancreatic

development. Furthermore, the neogenesis of islet β cells is achieved if both epidermal, as well as gastrin growth factors, are used to treat human islets (containing acinar and ductal cells), increasing the β cell population (Takahashi et al., 2007). Based on these findings, acinar and ductal SCs can be considered possible generators of new pancreatic islets (Saleem et al., 2019).

11.3.5 Hematopoietic Stem Cells

Bone marrow (BM) is regarded as an indispensable origin of adult stem cells that are readily available. Both ectodermal and endodermal differentiation is possible with these bone marrow-derived stem cells (BM-SCs) (Y. Jiang et al., 2002). Previous research has shown that the BM contains cells capable of differentiating into β cells (Ianus, Holz, Theise, & Hussain, 2003). In the generation of β cells from BM-SCs within an appropriate in vitro microenvironment, a variety of transcription factors are functioned (Moriscot et al., 2005). But, there is a lot of disagreement over the ability of these stem cells to differentiate on a broad scale (Wagers, Sherwood, Christensen, & Weissman, 2002). Studies have revealed that pancreatic regeneration is induced by BM-SCs (Hess et al., 2003).

Studies reveal that recipient insulin-producing β cells undergo neogenesis and fast proliferation when BM-derived SCs are transplanted into ducts and islets. According to the hypothesis of researchers, vascular pancreatic endothelial cells are differentiated from grafted BM-SCs. These cells invoke some unknown differentiation and growth factors, causing β cell reproduction from pancreatic host cells. However, several difficulties must be solved before this technique to be feasible to treat type 1 diabetes (Samli, 2019). According to recent research, the c-Met/hepatocyte growth factor (HGF) signaling pathway enhances pancreatic reproduction in receivers following bone marrow transplantation (Samli, 2019). Studies reveal that the capacity of adult human stem cells to generate autoantigen tolerance is achieved. DM-resistant class-II major histocompatibility complex (MHC) is expressed after autologous HSCs

are implanted. Central tolerance is enhanced through this transplantation, causing the defense against the onset of diabetes (C. Tian et al., 2004).

The presence of antigen keeps a role to monitor T-cell immunity, as evidenced by the researches (Garza et al., 2000). The presence of antigen by resting antigen-producing cells (APCs) resists T cells from responding and producing antigen-specific antibodies (Hawiger et al., 2001). Stem cells differentiate into APCs and lentiviral vector transduction of human SCs can successfully direct gene expression to MHC class II positive APCs in vivo (Cui et al., 2002). In addition, proinsulin also acts as the leading autoantigen which is important in β cell demolition and progression of T1DM (Krishnamurthy et al., 2006; Narendran, Mannering, & Harrison, 2003). It provides a way of targeting the expression of the proinsulin gene. This technique helps to prevent autoimmunity after the transplantation of islet cells (Saleem et al., 2019).

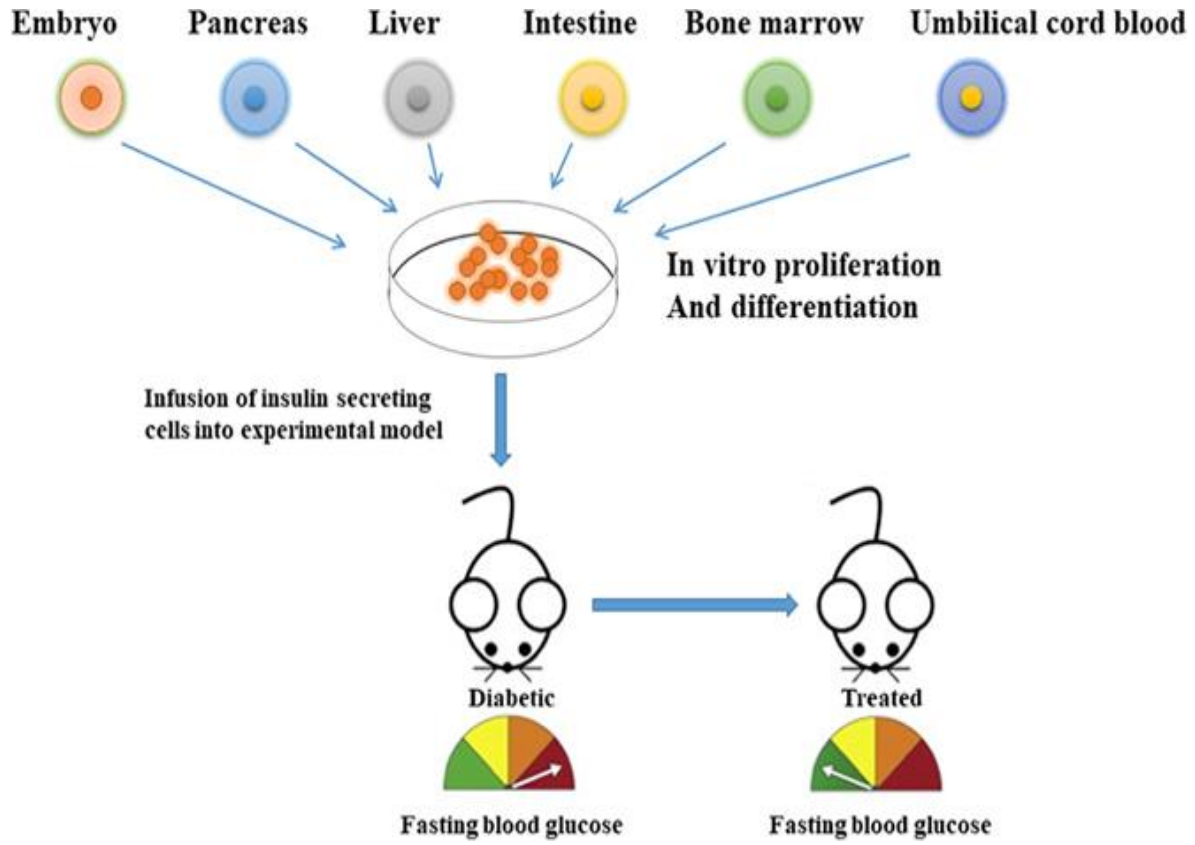


Figure 5: Differentiation of Several Stem Cells into Beta Cells, Adopted from, (Saleem et al., 2019)

Chapter 12

Basic Concept of Transcription Factors and Signaling Pathways

The majority of stem cell differentiation techniques aim to produce glucose-responsive, mature, and single hormone-expressing pancreatic β cells (Jørgensen et al., 2007; Murtaugh, 2007). Several signaling pathways and different types of transcription factors help to generate stem cells. The endodermal formation during gastrulation is involved with the transcription factors and homeobox gene HB9 (Hlxb9) and SRY (sex-determining region Y)-box (Sox)17 (Solis, Moreno Velásquez, Correa, & Huang, 2019). The endocrine pancreatic cells are developed, proliferated, and differentiated by inducing signaling pathways including retinoic acid, fibroblast growth factor (FGF)-10, hedgehog, TGF-beta, Notch, SOX9, and Wnt after foregut formation in which endocrine cell lineage is regulated by growth differentiation factor (GDF) and activin specifically. Both embryonic and adult stem cells are regulated by these signaling (Sarath & Rani, 2012; Scharfmann et al., 2008).

Pancreas-specific transcription factors include pancreatic duodenal homeobox-1 (PDX-1), neurogenin-3 (NGN-3), Ptf-1a, MafA and NK6 homeobox 1 (Nkx6-1) proceeds pancreas specification and budding process that allows the formation of endocrine and subsequent stimulation of NK2 homeobox 2 (Nkx2-2), paired box gene (Pax)4, ISL LIM homeobox 1 (Isl-1), Pax6 signaling and neurogenic differentiation factor (NeuroD) which form the islets of Langerhans. During the pancreatic development the transcription factors HNF-3beta (alternatively known as the forkhead A2, Foxa2), HNF-6, and Sox 17 are expressed. Lastly, neogenesis is stimulated through the differentiation of pancreatic progenitor cell, notch, and FGF-10 signaling-induced stem cell which results in the generation of β cells (Bhushan et al., 2001; Hald et al., 2003). The identification of core components involved in β cell development has resulted in ways to acquire β cells such as supplementing soluble components

to their cultures or triggering the production of pancreatic-related transcription factors in various sorts of stem cells (Solis et al., 2019).

The function of the adult islet, the proliferation of beta-cell and insulin gene expression via laminin are maintained by vascular endothelial growth factor (VEGF) (Mishra, Singh, Joshua, & Tyagi, 2010; Sarath & Rani, 2012). Transcription factors facilitate the sequential transformations in specialized endocrine precursors from uncommitted progenitors and then fully differentiated β cells in a progressive manner. Internal and external signals culminate in the transcriptional factors. Two key transcription factors such as pancreas transcription factor 1a (Ptf1a) and pdx1 are crucial at the primary state of pancreatic progression. These factors help to proliferate, differentiate and maturate pancreatic β-cells (Kaneto et al., 2008). In the development of pancreatic exocrine and endocrine parts, Pdx-1 is a significant marker that plays a vital role (Goldsworthy et al., 2008). Pdx1 is necessary for normal β cell homeostasis because it has a great contribution in the transactivation of the βcell-specific genes such as insulin, GLUT2, βcell-specific glucose transporters glucokinase (GCK), and IAPP (islet amyloid polypeptide) (Piper et al., 2002; Liew & Andrews, 2008).

Sox4 has been linked to the control of insulin secretion in adult β cells in recent years (Goldsworthy et al., 2008). Sox9 helps in regulating neurogenin3 positively and maintaining a pancreatic progenitor pool (Lynn et al., 2007; Seymour et al., 2007). It is mainly seen in the early stages of pancreatic development (before 14 weeks of gestation) (Liew & Andrews, 2008). Sox9 appears to be a determinant of multipotent pancreatic endocrine cells in the pancreas, according to a study employing sox9 heterozygous mice mutants (Liew & Andrews, 2008; Seymour et al., 2007b). The transcriptional regulator islet 1 (Isl-1) keeps an important role in the endocrine pancreas' proliferation, development, and survival (Du et al., 2009). Pancreatic progenitors are constituted through the stimulation of Sox, Ptf1, and Pdx1. For Ngn3 expressing islet progenitor cells, HNF1β

contributes to endodermal differentiation, pancreatic growth in the precursor population as a whole (Poll et al., 2006). At various stages of development, Ngn3 can create several endocrine cell lineages (Johansson et al., 2007).

Lineage traceability shows that cells of Pax4+ are involved in all endocrine lineages. Pax-4 is a factor that controls the number of beta cells in the body (Wang et al., 2008). The islet progenitors are constituted by Pax4, Ngn3, and HNF1β. Islet-cell growth, functioning of beta cells, and morphology are all dependent on Pax-6 (Mishra et al., 2010). MafA is a newly discovered transcription factor that regulates diabetes pathogenesis, as well as beta-cell growth and the exposure of the insulin gene on beta cells which is activated toward the end of the β cell differentiation process (Liew & Andrews, 2008; Sarath & Rani, 2012). Nkx6-1 is a β cell determinant, whereas Nkx2-2 is involved in beta cell ultimate differentiation which leads to the generation of insulin (Sarath & Rani, 2012). The second phase of the neogenesis of β cell in the growing pancreas necessitates the exposure of Nkx6-1 (Jensen, Serup, Karlsen, Nielsen, & Madsen, 1996; Liew & Andrews, 2008; Rudnick, Ling, Odagiri, Rutter, & German, 1994).

Several additional genes were also discovered to be necessary for the function of β cells. Several MODY genes are included in this list which are hepatocyte nuclear factor 4a (HNF4A; MODY-1), hepatocyte nuclear factor 1b (HNF1B; MODY 5), hepatocyte nuclear factor 1a (HNF1A; MODY-3), glucokinase (GCK; MODY-2), insulin promoter factor-1 (MODY-4) and NEUROD1 (MODY-6) (Liew & Andrews, 2008; Malecki et al., 1999).

Chapter 13

Materials and Methods of Embryonic Stem Cell Culture

13.1 Instrumentation

Hamilton Microlab STAR workstation serves as the foundation for the embryonic stem cell cultures (Cellhost) system that has been modified to meet the needs of cell culture. To evacuate the supernatant during media transformations, a specific plate lifter is implemented for tilting cell culture plates. During the cell plating process, the robotic arm was modified to mimic manual actions. This procedure has been adapted for the homogenous plating of single-cycle ESC aggregates according to various requirements. In addition, to trypsin-treatment and gelatin-coating, a heat shaker module (CAT, Staufen, Germany) has been installed. To warm up a cell cultivation media, heating elements are needed.

Figure 6: Hamilton's Cellhost System, Adopted from,(Terstegge et al., 2007)

The complete system was designed to operate under the controlled air displacement technology based on pipetting principle. A washing plant for reused pipetting needles in conjunction with a Millipore water purification system has been installed to limit disposables expenditures. The

automated system contains neither tubing nor system fluid as well as this washing station, which prevents contamination. A sterile enclosure with a UV decontamination system and a laminar air flow and contains the pipetting station. The pipetting platform is connected with two robotic accessible incubators for media (capacity of 20 media tubs, 48°C) and cell culture plates (five percent CO2 atmosphere, 378°C, receptivity of 153 SBS format multiwall cell culture plates) storage by two linear transfer units. Barcode readers are installed in both incubators. A user-specified media tub is typically transported from the incubator to the heating organ and heated to 378°C. After that, the user selects a cell culture plate and transports it from the incubator to the pipetting station. The Cellhost assures that the media tub carries enough media for the complete plate media shift by using the liquid level detection technology. By using the plate lifter, all used media is dispelled from the plate and new media is added. The plate trail has been updated with information on the data, operated process, media tub barcode, user and media tub lot number while the media tub and cell culture plate are placed back to the incubators (Terstegge et al., 2007).

13.2 Plate Tracking

To track in Hamilton's CellTrack database, barcode-labeled plates are employed on the systems. All plates on the system are recorded in a plate list which provides access to information such as the most recent status change dates, plate status regarding the process of cell culture, and the plate barcode. In addition, each processing phase of a single plate such as lots of media, operator information, date and time, and handheld activities like microscopic cell culture analysis are documented in plate trails (Terstegge et al., 2007).

13.3 Culture of Stem Cell

The isolation and culture methods of human embryonic stem cells (hESC) are usually carried out by following the same cultivation process as mouse embryonic stem cells. A media enriched with inactivated mouse embryonic fibroblasts (MEF)-based feeder layers and fetal bovine

serum (FBS) are employed in this process (Thomson, 1998). However, current research has focused on the process of cultivating hESC without the necessity of using a MEF-conditioned medium to support feeder layer to grow cells in serum-free conditions (C. Xu et al., 2001; C. Xu et al., 2005; Michal Amit et al., 2000; M. Amit, Shariki, Margulets, & Itskovitz-Eldor, 2004; R.-H. Xu et al., 2005; Sperger et al., 2003). Undifferentiated hESC can be cultivated by using suspension cultures where serum-free medium with Bfgf and full-length IL6RIL6 chimera are used to promote the culture in the suspension of multiple hESC lines which is an effective way of stem cell culture. Mouse embryonic fibroblast is used to grow suspension cultures with IL6RIL6 and bFGF factors.

Several growth factors and cytokines are employed to induce HESC suspension cultures cultivated under feeder-dependent conditions (M. Amit et al., 2004). The hESC generates disc-like formations or spheroid clumps, 24 hours after being put into suspension culture with the IL6RIL6 chimera (CH100F). Suspended cells, disassociated into single cells, continue to produce spheroids with the same characteristics and morphology, enabling rapid cellular proliferation after being treated with ROCK inhibitor and trypsin–EDTA. After 10 and 25 suspended passages, MEF or fibronectin are re-cultivated and all spheroids adhere to MEF or fibronectin, and morphology similar to that of HESC colonies is presented 24–48 hours later. 85 percent DMEM/F12 with 2 mM L- glutamine, 1 percent non-essential amino acid stock, 15 percent knockout serum replacement, 4 ng/ml bFGF, and 0.1 mM β–mercaptoethanol makes up the basic culture media. This basic culture media is utilized for the routine growth and controlling of hESC in 2D cultures with MEF feeder layers (Michal Amit et al., 2010).

In short, hESCs are broadened for G-irradiated primary mouse embryonic fibroblast (MEF) derived from mouse strain CD1 in culture media consisting of KnockOut DMEM supplemented with twenty percent KnockOut Serum Replacement, 4 ng/mL FGF-2, 1 mM glutamine, and 1% non-essential amino acids. Culture media is renewed every day and hESC

is subcultured every four to five days using 1 mg/mL collagenase type IV. Every day, the culture media is replaced and 1 mg/mL of type IV collagenase is being used to subculture stem cells every four to five days. The daily monitoring of the cell's lactate generation and consumption of glucose is carried out. Immunocytochemistry is applied to examine EBs after 3 weeks of suspension culture (Terstegge et al., 2007).

Chapter 14

Differentiation of Pluripotent Stem Cells into Insulin-secreting β Cells

Effective management of culture conditions and the incentives for core regulatory genes connected in the evolvement of the pancreas are necessary for successful β cell production from pluripotent stem cells (PSCs). Pluripotent stem cells, which comprise ESCs and iPSCs can develop into cells of all three germ layers and have a wide range of self-renewing capabilities. The hESCs and hiPSCs have identical properties and can differentiate, utilizing the same methods into any form of cell in the body such as pancreatic β cells (Chen et al., 2009; Maehr et al., 2009). PSCs can be developed into insulin-secreting β cells through remodeling the culture conditions, employing an embryo body (EB) technique or monolayer culture according to the previous research (Kroon et al., 2008; Shim et al., 2007). Since the cell density of initial seeding influences the effectiveness of differentiation into pancreatic endocrine cells, the first stage of human embryonic stem cell differentiation is essential for optimal pancreatic differentiation according to a prior study.

The high-density seeded hESCs are more capable than low-density seeded cells to differentiate into neurogenin 3 (NGN3)-positive cells and pancreatic duodenal homeobox 1 gene (PDX-1) (Moncada, 1993). Previous studies have shown that up to 12 percent of insulin-secreting cells can be differentiated from hESCs, albeit with poor reactivity to glucose (D'Amour et al., 2006; Jiang et al., 2007). Currently, it was discovered that the family members of transforming growth factor β (TGFβ) help to produce up to 25 percent insulin-secreting cells from hESCs with better effectiveness through in vitro management (Nostro et al., 2011). Initially, the PSCs are differentiated to definitive endoderm (DE) as occurs in vivo as a preliminary step towards pancreatic differentiation. Recent DE differentiation protocols from hESCs have demonstrated that at least sixty to eighty percent of differentiated cells express FOXA2, GSC, CXCR4 and SOX17, but they do not explicit SOX7 which is the visceral endodermal marker. The NODAL

and WNT signals are found to be essential signals for inducing DE from hESCs. A NODAL signal is activated with activating A which is an endogenous endoderm inducer (T. Tian & Meng, 2006).

The high levels of activin A (50-100 ng per ml) in the lack of serum are indicated to be required for efficient DE induction from hESCs. In addition, DE differentiation is stimulated by the treatment of PSCs with antagonists of the PI3K pathway or a combination of activin A and sodium butyrate (McLean et al., 2007). The effectiveness of the differentiation of DE is also boosted by the treatment of hESCs with Wnt3A or bone morphogenetic protein 4 (BMP4) (D'Amour et al., 2005). DE differentiation increased by adding CHIR99021 or WNT3A which activates Wnt signaling through inhibiting glycogen synthase kinase 3 β (GSK3β) during activin therapy. GDF8 (myostatin), a member of the TGF family, is also efficient in inducing DE (Hosoya, 2012). Nearly eighty percent of ESCs differentiate into SOX17-expressing DE-cells through hESC treatment with small molecules such as inducer of definitive endoderm 1 (IDE1) and inducer of definitive endoderm 2 (IDE2) (Borowiak et al., 2009). Additional growth factors in culture media need to be added for further differentiation of DE cells into pancreatic β cells (Mfopou, Chen, Mateizel, Sermon, & Bouwens, 2010). Pancreatic progenitor cells are treated with hepatocyte growth factor (HGF), insulin growth factor 1(IGF-1), glucagon-like peptide 1 (GLP-1), dexamethasone (a synthetic adrenocortical steroid) and forskolin (an adenylate cyclase activator) to improve the maturation of pancreatic β cell (Abdelalim, Bonnefond, Bennaceur-Griscelli, & Froguel, 2014; Ohmine et al., 2012).

Chapter 15

Patient Specific-Embryonic Stem Cells Therapy in Diabetes mellitus

Converting embryonic stem cells into pancreatic cells, which can subsequently be replanted into the donor's body, is possible. Personalized ESCs from a patient's somatic cells can be generated from the process of therapeutic cloning which is entitled somatic cell nuclear transfer (SCNT). SCNT technology comprises transplanting the nucleus of a somatic cell into an egg cell that has had its nucleus removed, resulting in embryos that nearly correspond to the parental somatic cells. SCNT was the sole system of generating patient-specific ESC lines for a long time that could be utilized to study the pathways of disease and autologous transplantation (Lanza, Cibelli, & West, 1999; Yang et al., 2007). Five to seven days after completing the process of SCNT, hESCs are produced from the internal cell mass (ICM) of a blastocyst stage. These obtained patient-specific hESCs can be developed into a variety of cell types, comprising insulin-secreting cells, which can be employed in disease modeling and cell therapy. The first mammal 'Dolly the sheep' was invented in 1997 using SCNT technology (Wilmut, Schnieke, McWhir, Kind, & Campbell, 1997). For several years it did not work in humans. Using the SCNT method, human somatic cells have successfully converted into hESCs in a recent study to overcome past difficulties (Abdelalim et al., 2014; Tachibana et al., 2013).

Chapter 16

Patient Specific-Induced Pluripotent Stem Cells in Diabetes Mellitus

As human embryonic stem cells have limitations in terms of disease modeling, researchers have concentrated on alternative options, including the newly discovered iPSC technology (R. Maehr, 2011). The production of iPSC lines from patients with various kinds of diabetes has recently been documented in several publications. Three transcription factors such as KLF4, SOX2, and OCT4 are used to produce the first hiPSC lines from T1DM patients' skin fibroblasts (René Maehr et al., 2009b). To model the disease in vitro, hiPSC lines have recently been originated from monogenic diabetes MODY. hiPSCs have been produced from MODY2 patients by Hua et al. that are characterized by mutation of the gene to encode glucokinase (GCK) (Estalella et al., 2007; Froguel et al., 1993). Then, insulin-secreting β cells are differentiated from these hiPSCs produced from MODY2 patients. β Cells in MODY2 individuals with GCK mutations can react to glucose, although their sensitivity is poor (Byrne et al., 1994). On the other hand, MODY2-specific iPSCs that have been corrected with the GCK gene can develop into pancreatic cells with normal glucose sensitivity (Hua et al., 2013).

In a further study, hiPSC lines have been successfully produced from a patient with various forms of MODY including MODY1 (HNF4A), MODY3, MODY2 (GCK), MODY8 (CEL), and MODY5 (HNF1B). For reprogramming, this research used a polycistronic lentiviral vector which was more efficient than commonly employed retroviruses, and the resulting MODY-hiPSCs showed no karyotypic abnormalities (Teo et al., 2013). HiPSCs might be generated from epidermal keratinocytes of T2DM patients aged 56 to 78 under feeder-free/serum-free culture conditions, according to recent work. The keratinocyte-derived iPSCs from diabetic and non-diabetic patients are very comparable to hESCs and can develop into all lineages, including insulin-secreting cells. iPSCs generated from diabetic and non-diabetic patients are comparable to hESCs and can develop into any lineages such as insulin-secreting cells (Ohmine

et al., 2012b). The use of viral transfection of transcription factors allows somatic cells to be reprogrammed into iPSCs.

Additionally, further studies have shown that iPSCs can be generated without the use of viruses. Moreover, they can be generated through the direct supply of recombinant proteins of reprogramming factors (Kim et al., 2009; Zhou et al., 2009). Non-integrating Sendi viral vectors are used to produce transgene-free hiPSCs from patients with T1DM and T2DM (Kudva et al., 2012). Recent strategies for generating iPSCs free of viral transgenes have reduced genome modification in iPSCs, aiding the development of iPSCs for cell therapy and disease modeling. Somatic cells can be reprogrammed by being exposed to sublethal stimuli such as a transient low pH, as Obokata and colleagues discovered, and they dubbed this phenomenon stimulus triggered acquisition of pluripotency (STAP). Contrary to ESCs, both embryonic, as well as placental tissues, can be produced by the STAP cells. Surprisingly, no genetic manipulation or nuclear transfer is requisite for this procedure (Abdelalim et al., 2014; Obokata et al., 2014).

Chapter 17

Mesenchymal Stem Cell Therapy in Diabetes Mellitus

When compared to other stem cells like embryonic stem cells or stem cells from other organisms, mesenchymal stem cells (MSCs) have a significant advantage. These cells are spindle-shaped fibroblast-like cells that can differentiate into three different cell types. MSC-derived functional cells may also not be rejected when transplanted into MSC donors (autologous transplantation). Due to its multi-potentialities, MSCs are shown to achieve adequate cells in treatment which include pluripotency, autonomy, reduced toxicity, facilitate culture and in vitro expanding, and low antigenicity. Several areas of our body such as bone marrow, amniotic fluid, adipose tissue, the placenta, and umbilical cord blood where these cells can be found. MSCs generated from the placenta and adipose tissue can be extended for multiple passages without losing their ability to self-renew. Friedenstein et al. were the first to isolate them from rat bone marrow, and they have since been discovered in a variety of different tissues.

The absence of endothelial and hematopoietic markers (HLA class II molecules, CD45, CD14, CD31, CD34), as well as the presence of specific surface markers (HLA class I molecules, CD90, CD105, CD73, CD29 are used to characterize these cells. These are simple to spread and take about 24–48 hours to double their population. MSCs are most commonly seen in the bone marrow that have been employed for development into cells of all three lineages in vitro. Other light invasive sources of MSCs such as umbilical cord, adipose tissue, and dental pulp have been investigated in the previous decade. The optimal cell source should be simple to expand, harvest, have a large supply, and be capable of producing functional β cells. Chemical methods and genetic interventions can both be used to develop MSCs into insulin-secreting functional β cells. According to Oh et al., BM-MSCs can produce insulin-secreting cells when exposed to high glucose concentrations

and Dimethyl sulfoxide (DMSO). For the production of functionally active insulin-secreting cells that could manage hyperglycemia in diabetic rats, Chen et al. employed nicotinamide and 2-mercaptoethanol (BME) under low glucose conditions in 2004 (L.-B. Chen, Jiang, & Yang, 2004).

Furthermore, Tang et al. employed exendin-4 and nicotinamide to isolate, define, and develop a mouse bone marrow stem cell line into insulin-producing cells from single cells. In a custom-made serum-free medium consisting of a basal medium supplemented with retinoic acid, platelet lysate (PL), GLPI-1, activin, fibroblast growth factor (FGF), epidermal growth factor (EGF), nicotinamide, glutamine, and betacellulin for 3 weeks. Zanini et al. compared human bone marrow-derived mesenchymal stem cells (BM-MSCs) to islet differentiation of human pancreatic islet-derived mesenchymal stem cells (Human islet mesenchymal stem cells: HI-MSCs). Adipose tissue has emerged as the most abundant source of MSCs in the previous decade. Because of the simplicity of isolation and abundance, the use of adipose tissue in stem cell therapy has attracted a lot of attention. Apart from adipose tissue and bone marrow, the placenta and the umbilical cord (Wharton's jelly and blood) are new sources of MSCs that have been discovered to be capable of differentiating into islet-like cells. Chemical signals such as high glucose media supplemented with mostly nicotinamide, BME, EGF, FGF, exendin-4, and retinoic acid are used to differentiate them (Sun & Ji, 2009). Several studies have demonstrated that cells obtained from umbilical cord blood can be coaxed into generating insulin (Kakkar et al., 2018; Solis et al., 2019).

Chapter 18

Advantages of Stem Cell Therapy

Regenerative medications and therapeutic cloning are two sectors where stem cell therapy offers medical applications. This therapy holds significant promise for developing treatments and cures for diabetes mellitus. Organs and limbs can be cultivated from stem cells in the laboratory, then utilize for transplantation and treatment of ailments. Through stem cell therapy-based research, scientists will get a better understanding of cell formation and human growth. After that, the benefit of adult stem cells is that they can use the patient's own cells to treat diseases. Patients' bodies would not refuse their cells themselves; hence the hazards would be greatly diminished. In addition, it is possible to differentiate embryonic stem cells into any cell lineage of the body and to be more versatile than adult stem cells (Elton, Roveena, & Manish, 2018).

Chapter 19

Limitations of Stem Cell Therapy

Although there are numerous efficacies associated with stem cell therapy, it includes some limitations too. Blastocysts are destructed after fertilizing human eggs in the laboratory when embryonic stem cells are needed for research. Destroying this blastocyst is immoral and unethical according to their aspect who believe that life begins at the moment of conception and the blastocyst is a human life. Then, not every malady can be solved by embryonic stem cells. Most adult stem cells have already been specialized, meaning that blood stem cells only generate blood and brain stem cells only generate brain cells alike, which is another limitation of stem cell therapy (Elton et al., 2018). In addition, adult stem cells do not have the ability of pluripotency. For all organs, they may not exist. Purifying adult stem cells is not an easy process and they are also hard to grow in culture without just a few exceptions (Miszta-Lane, Mirbolooki, James Shapiro, & Lakey, 2006). Moreover, teratoma which is a tumor-like structure can be formed by embryonic stem cells (Choumerianou, Dimitriou, & Kalmanti, 2008).

Stem cell types	Sources	Benefits	Drawbacks
Embryonic stem cells	Human blastocysts	Pluripotent	It necessitates the demolition of embryos.
Adult stem cells	Adult tissues	Multipotent	These cells are available in fewer places in the human body. In addition, These cells can only regenerate in tissues that are identical or related to the ones from which they are derived, hence their application is restricted.
Fetal stem cells	Gonads of aborted fetuses	Multipotent	It necessitates destroying the fetus that is only a few weeks old.
Induced pluripotent stem cells	Adult tissue cells are reprogrammed to become pluripotent.	Pluripotent	They are not patentable.
Umbilical cord stem cells	The umbilical cord blood of neonates	Multipotent/ pluripotent	It has a low frequency.
Placenta-derived stem cells	Placenta of neonates	Multipotent/ pluripotent	The frequency of this type of stem cell is low but higher than cord blood.

Table 4: List of Sources of Stem Cells with Their Advantages and Disadvantages (Elton et al., 2018)

Chapter 20

Encapsulation of Stem Cells

In islet transplantation, the encapsulation technique is currently used for protecting against the immune system of the host and improving graft function. DM is effectively treated using encapsulated SCs (Niknamasl et al., 2014). Encapsulation of pancreatic SCs present in growing pancreatic buds is done using biologically inert and polyethylene glycol-based hydrogels. This encapsulation method removes both the extracellular matrix and the heterogeneity of cell signaling, all of which are required for significant β cell generation. Encapsulated stem cells exhibit selective differentiation into insulin-producing β cells as well as long-term survival (Hashemi & Kalalinia, 2015). Because of the biocompatibility of alginate, it has been used extensively which is a scaffolding polysaccharide generated by brown seaweeds. Alginates are unbranched linear polymers and they are made up of α-(1 → 4)-linked L-guluronic acid residues and β-(1 → 4)-linked D- mannuronic acid.

If polyvalent cations such as Ba2+ and Ca2+ are present, alginates show excellent gel-forming capabilities. With the use of purified alginate, encapsulated islets have long-term function and biocompatibility, significantly increased survivability compared with non-encapsulated islets (Shuai Chen et al., 2020). Human umbilical cord blood and BM-SCs were first differentiated into β cells before being encapsulated in a membrane of alginate. Then, they were grafted with intraperitoneal injection into a diabetic patient. Graft rejection was prevented by encapsulating allograft or xenograft β cells (Ngoc, van Phuc, Nhung, Thuy, & Nguyet, 2011). By generating prolonged local immune isolation, the chemokine CXCL2 combines with alginate microcapsules to avoid immune reactions after allo- or xenoislet transplantation. Glucose stimulated insulin secretion (GSIS) activity of β cells is increased through CXCL2 which makes it a vital biomaterial for stem cell-based DM therapy (Shuai Chen, Du, & Zou, 2020).

Encapsulation with fibrin hydrogels has also been attempted. The characteristics of fibrin hydrogels are similar to the normal pancreas. According to the findings of many kinds of research, the cells encapsulated in fibrin hydrogels have good integrity and produce islet cell clusters that generate insulin after two weeks (Niknamasl et al., 2014; Saleem et al., 2019). Several factors should be taken into account while assessing an encapsulating device. Those factors include stability, biocompatibility, oxygen, permselectivity of the membrane, availability of nutrients, and interaction with the bloodstream (Shuai Chen et al., 2020).

Advantages	Limitations
• Fast cell growth and cell durability are promoted • Cells are protected from the immune system of the host • Suitable for cryopreservation • Ensure mechanical stress safeguards • Controlled and well-managed supplies are maintained • Cell differentiation is improved	• Look for an appropriate site of transplantation • High reproducibility and capsule consistency • Encapsulated cells require nutrition • Capsule stability • Polymer-encapsulated cell interactions • Controlling encapsulated cell's growth, culture, and differentiation • Polymer characteristics, both chemical and physical • Immunogenicity and toxicity

Table 5: Advantages and Limitations of Encapsulation Method of Differentiated β Cells Transplantation (Saleem et al., 2019)

Chapter 21

Immune Modulation in Stem Cell Therapy

As a potential source of β cells substitution to treat diabetes mellitus, human β cells derived from embryonic stem cells/induced pluripotent stem cells are used. But, the extensive use of DM cell replacement therapies still has major problems in both alloimmune and autoimmune responses. Despite significant efforts in the advancement of encapsulation technology, engraftment of transplanted hPSC-derived pancreatic beta or progenitor cells faces difficulty. When the encapsulation system is eliminated, the engraftment has perished through the immune system of the receiver. Some modulations of these encapsulated cells appear to be beneficial in terms of preventing autoimmune attacks. Immunological rejection in allo- or xenografts is caused by mismatching of the human leukocyte antigen (HLA) (Williams, Opelz, McGarvey, Weil, & Chakkera, 2016).

Studies have shown that donor compatibility can be improved by removing HLA-A genes from hematopoietic stem cells using zinc-finger nucleases (Torikai et al., 2013; Torikai et al., 2016). Also, when the β2 microglobulin (B2M) gene is knocked out, that prohibits all HLA class I molecules, or that biallelically removes HLA-A and HLA-B, the one allele of HLA-C is retained so that the NK and T cell attack can be prevented by the hPSC grafts (H. Xu et al., 2019). The establishment of DM and allo-islet rejection is effectively suppressed through the targeted overexpression of PDL1- CTLA4Ig in cells, which is the other immunosuppressive method that has been discovered (El Khatib et al., 2015). As a result, the techniques of immune modulation for hPSCs may offer solutions in overcoming engraft rejection problems (Shuai Chen et al., 2020).

Chapter 22

Clinical Trials in Stem Cell Therapy

The safety and efficacy of stem cells therapy to treat type 1 diabetes mellitus were assessed through controlled clinical trials in recent years. In animal models of T1DM, MSCs have been shown to alleviate or reverse the appearance of diabetes. MSCs therapy could retain the function of β cells in patients with new-onset T1DM, according to Carlsson et al. in 2014. For one-year follow-up assessment, twenty recently diagnosed (within the last three weeks) adult patients (18–40 years old) were registered and randomly assigned to either control group or MSCs treatment (Carlsson, Schwarcz, Korsgren, & Le Blanc, 2015). Mixed-meal tolerance tests (MMTTs) demonstrated that the treatment group had considerably lower C-peptide peak values and C-peptide levels at the end of the clinical study.

During follow-up evaluations, no side effects of MSCs treatment were found. Forty-two patients aged 18–40 years old with T1DM for at least two years and the highest 16 years were randomly assigned to one of two groups which are conventional insulin treatment or stem cell transplantation (autologous bone marrow mononuclear cells in combination with umbilical cord MSCs) during January 2009 and December 2010 (Cai et al., 2009). The C-peptide reduced from 8.4 to 7.7 pmol/mL/180 min in control groups while it was increased from 6.6 to 13.6 pmol/mL/180 min in treated patients and insulin declined from 1517.7 to 1431.7 mmol/mL/180 min in control patients while it was increased from 1477.8 to 2205.5 mmol/mL/180 min in treated patients, according to one-year follow-up examination. Fasting glycemia and HbA1c level also increased in the control groups while dropping in the treated groups. In addition, regular demands of insulin in the treated categories were lower than control categories requirements. Patients' reports of severe hypoglycemia incidents reduced considerably during the follow-up period. These findings will contribute to the enhancement of clinical trial outcomes in large-scale trials in the future (Shuai Chen et al., 2020).

Chapter 23

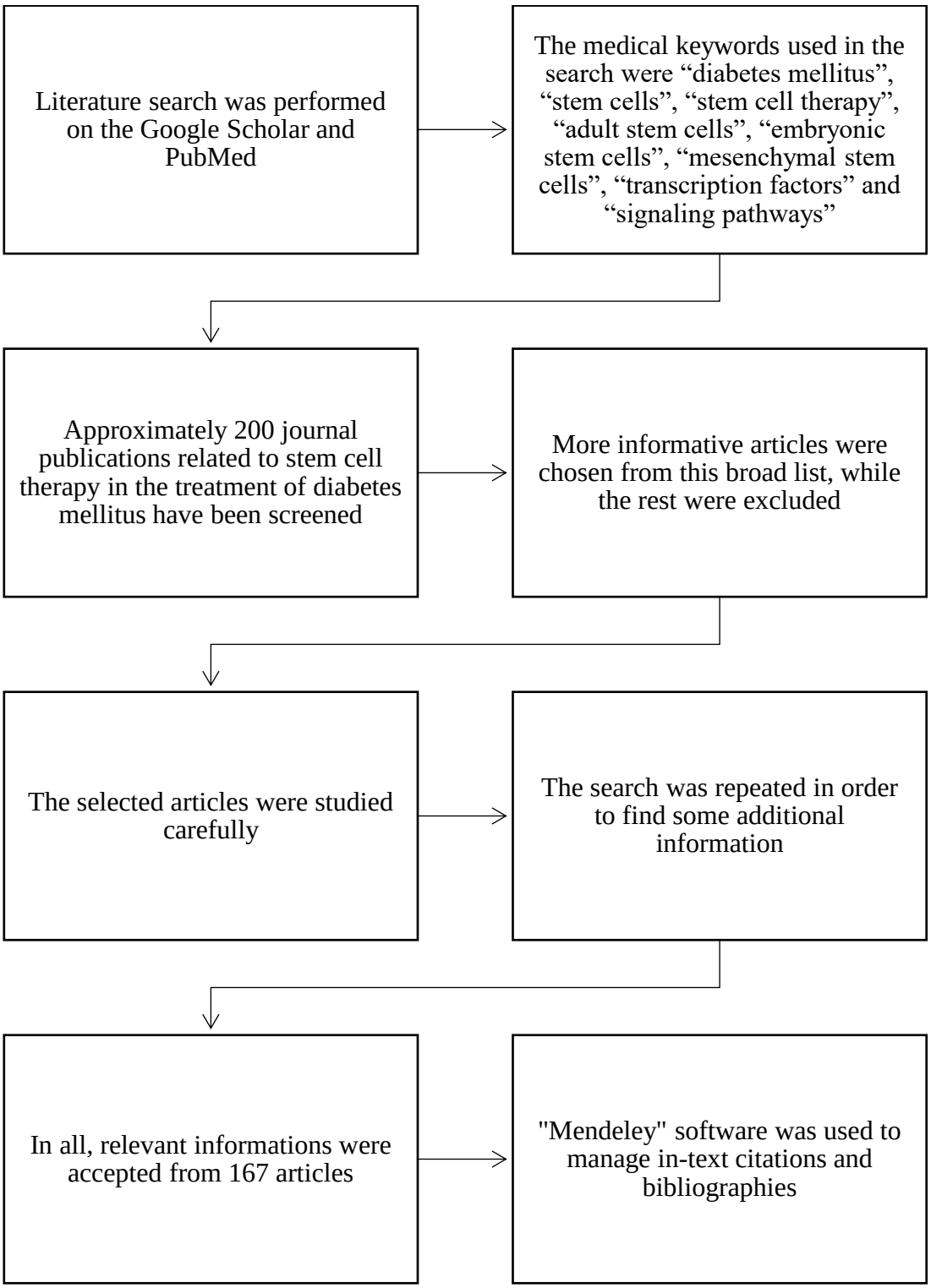
C-Peptide Responsiveness after Stem Cell Therapy

C-peptide, a surrogate marker for endogenous insulin secretion, represents this secretion. The reaction of C-peptide can be measured by measuring serum C-peptide levels in the fasting state and during the stimulation procedure. Fasting blood glucose (FBG) controls the level of C-peptide. The concentration in the HBA1C, the exogenous insulin dose, stimulated patterns of C-peptide change, and fasting C-peptides must all be taken into account at the same time to assess locally injected treatment for stem cells has increased the function of β-cell for diabetic patients.

After stem cell therapy, four key mechanisms for C-peptide responsiveness might be considered. At first, Pancreatic beta cells can be differentiated through injected stem cells. In vitro, ESCs develop functional beta cells. Secondly, progenitor cells are recruited with the ability to develop into beta cells through paracrine action, which results in the production of new beta cells. The hyperglycemic condition of the diabetic patient becomes normal and beta-cell mass is regained when a diabetic patient is intravenously injected with an enhanced green fluorescent protein (eGFP) marked adult stem cells. Thirdly, enhancement of anti-inflammatory cytokines and reduction of pro-inflammatory cytokines safeguard the remaining beta cells and make them easier to repair and proliferate. In addition, the C-peptide level is increased, the cytokine equilibrium between T helper cells is recovered and the number of regulatory T cells is increased in diabetic patients after ‘Stem cell educator' therapy which involves reinjecting lymphocytes after educating them into human cord blood-derived multipotent stem cells in vitro. Fourthly, autoimmunity is inhibited and therefore the destruction of residual beta cells is prevented and diabetes progression is slowed down or inversed (Hwang et al., 2019).

Chapter 24

Methodology



Chapter 25

Discussion

It has been shown that the rate of diabetes prevalence has increased globally in recent decades. Diabetes was accounted to affect 451 million individuals globally between the ages of 18 and 99 according to the statistics of 2017. Based on previous statistics, it was assumed that the number of diabetic patients will increase to 693 within 2045. Approximately five million people between 20 and 99 years of age died from diabetes worldwide. The rate of diabetes incidence among both men and women is expected to rise to 9.9 percent by 2045 (Cho et al., 2018). According to the research of Mohiuddin in 2019, the number of diabetes sufferers is ten million in Bangladesh (Mohiuddin, 2019). Studies have found a variety of causes of diabetes (Adeghate et al., 2006). Both environmental and genetic factors are responsible for the onset of this condition (Kommoju & Reddy, 2011) such as lack of physical activity, obesity viral infections, etc. (Adeghate et al., 2006).

Several methods have been developed to control diabetes up to now. The traditional approach is through exogenous insulin injections among these. However, it has been revealed that insulin therapy is unable to adequately manage blood glucose levels. As a result, patients suffer from a long period of consequences. Hypoglycemia can be raised due to the side effect of insulin treatment (Vija et al., 2009). At that moment, the advancement of pancreas transplantation offers some hope for the healing of diabetes. However, this approach has not been very efficient. Two severe inconveniences had been observed after pancreatic transplantation which are host immune rejection and restricted number of donors. Researchers have therefore investigated stem cell transdifferentiation as a potential source of beta cells (Sarath & Rani, 2012). There are several types of stem cells.

Studies have shown that stem cell transdifferentiation to produce pancreatic beta cells is regulated by signaling pathways and various transcription factors. Transcription factors help to carry out stem cell cultivation. Stem cell therapy offers a lot of potential in the field of creating diabetes treatments and cures due to their ability to develop into beta cells and immunomodulatory properties (Zhang et al., 2020). Despite the benefits of stem cell transdifferentiation, there are still some adverse effects and hazards with this therapy. It is known from various studies that several unfavorable effects are reported such as teratoma formation which is like a tumor when ESCs are transplanted into humans following differentiation. Transplanting completely developed, pure cells derived from hESCs is the best way to resolve this problem.

Additionally, the development of beta cells in the pancreas and through in vitro differentiation of stem cells face two different environments. For this reason, there are two types of obstacles to be faced after stem cell transdifferentiation. One is beta cells can be destroyed because of autoimmunity in T1DM and another one is the alloimmune response between the host and the graft can be triggered as the immune system perceives transplanted tissue as foreign tissue. Autoimmunity must be suppressed for cell replacement therapy to be successful through graft survival prolongation, enhancement of insulin, and control of glycemia (Saleem et al., 2019). The necessity for fresh grafts (typically within eight hours of death), the scarcity of donors, the risk of graft rejection, and the requirement for tissues from multiple donors for a single recipient all impede this therapy.

After that, the ethical issue of using embryonic stem cells has made endless controversy due to their source of origin. ESCs are generally produced from unfertilized embryos. ESCs, therefore, need to be obtained after destroying the embryo. As adult stem cells have no significant debate around their use, they can be used instead of ESCs (Saleem et al., 2019). Insulin-dependent individuals have a lot of optimism with these cells. Because of the easy

preparation process, simplicity of isolation, ability to differentiate into cells of many lineages, and immunomodulatory functions, MSCs are the most appealing options among adult SCs. These are the most well-studied stem cell types, with reports of them being used in the highest possible clinical trials (El-Badri & Ghoneim, 2013). To determine the optimum stem cell method for human patients, characteristics such as suitable cell dose, stem cell types, and mode of administration must be considered. Despite all of these difficulties, stem cell therapy has created its clinical prospects and shown its ability to substantially ameliorate the lives of millions of diabetes-affected individuals.

Chapter 26

Conclusion

Both type 1 diabetes mellitus and type 2 diabetes mellitus are among the most treatable disorders. Nowadays, the treatment of DM aims to reduce high blood glucose levels while limiting the complications of DM. Stem cell therapy can provide relief from the symptoms of the disease for people living with diabetes. To achieve the best outcome, every step from stem cell collection to administration must be done with extreme caution. Despite all the good aspects of this therapy, patients still suffer from several side effects after stem cell transplantation which are obstructing the progress of this therapy. Teratoma formation has been observed in some cases after stem cell transplantation which is such a major barrier. More researches must be carried out to promote the field of stem cell therapy and to better understand its potential. Despite the challenges, this therapy offers the most advanced method to diabetes treatment. By lowering diabetes-related problems and halting the development of the disease, stem cell therapy can greatly enhance a patient's quality of life.

Chapter 27

Future Work

Several studies have found that using stem cells in cell-based therapy to treat diabetes mellitus carries some potential risks. Using stem cells to establish a renewable source of beta cells for diabetes treatment is difficult, owing to safety issues. The phenomenon of forming teratoma after ESCs transplantation is a major concern that must be controlled in the future. To detect the adverse effect of using stem cells, careful screening and testing are needed. In addition, to overcome these difficulties, laboratory methodologies such as cell culture conditions and cell enumeration must be improved. Furthermore, anti-graft rejection drugs need to be ameliorated and administered to prevent graft rejection. To confirm future advances in the treatment of diabetes, more clinical trials on stem cell therapy and researches utilizing larger and better-controlled experiments are needed. In a word, the field of research should be expanded and the shortcomings must be rectified on stem cell therapy for the treatment of DM to ensure that life-saving therapy is not life-threatening.

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