2. AIM AND OBJECTIVES

Diabetes mellitus is a life-threatening and devastating disease affecting millions of people across the world. Maintaining good glycaemic control with exogenous insulin therapies a hardship on both type-I and type-II diabetic patients. Transplantation of total pancreas or isolated pancreatic islets is an attractive approach for treating type 1 diabetes patients. Still, the scarcity of cadaveric pancreas and the essential use of immunosuppressive drugs are restricting aspect. Capable of overcoming this is where different types of stem cells inaugurate new avenues for treating diabetes. Studies of embryonic stem cells show a promising role in treating disease but they are still remaining with controversial ethical issues. Strategies in diabetes therapy including genetically manipulated pancreatic β-cells and the use of pancreatic progenitors has their own limitations due to the slow turnover rate of β- cells culminating in inadequate treatment. Therefore, recent research is focused on increasing pancreatic islets mass from various stem cell sources using chemically synthetic or biological differentiating agents. More specifically, stem cell therapy using autologous adult mesenchymal stem cells has been documented for its potential to revolutionize the field of "Islet Biology". Of the various sources of adult stem cells, bone marrow (BM) has been the most frequently preferred source and it is widely used for clinical transplantation in the treatment of a wide range of life-threatening diseases. The main focus of this research is to explore the capability of human bone marrow-derived mesenchymal stem cells (hBMSCs) to differentiate into islet-like cell clusters (ILCCs), as to advocate the use of hBMSCs for autologous stem cell therapy for treating type 1 or chronic type II diabetic patients. hBMSCs are free of ethical and immunological complications, thus provide an unprecedented opportunity as starting stem cell material to derive insulin-producing cells and an ideal candidate for prospective stem cell therapy to treat diabetes.

Medicinal plants are a gift of nature to mankind, providing potent, safe, and better therapeutic options for treating various diseases including diabetes. Our lab reports suggested that, swertisin, a potent bioactive small molecule (isolated from *Enicostemma littorale* plant) with islet differentiating potential whose mechanism has been earlier stated by our lab in both *in vitro* as well as *in vivo* models. Hence, in the present study, we hypothesized that differentiating functional islets from hBMSCs can be achieved by exploring various bioactive molecules like swertisin, curcumin, etc. Thus, in the current study, we attempted to develop a novel islet differentiation protocol using bioactive molecules cocktail (BMC) from hBMSCs. Pancreatic transcription factors and microRNAs change their degree of expression throughout the islet

differentiation and pancreatic development. So, we were looking forward to examining the involvement of pancreatic transcription factors and microRNAs during islet differentiation of hBMSCs to ILCCs.

Another significant part of the current study was to improve encapsulated rat/ILCCs viability using key bioactive molecules, such as swertisin, curcumin, and genistein (*In vitro* study). Further, we developed a unique protocol for rat islet encapsulation for "Rat to Mouse" xenotransplantation study using novel hollow fibre membrane (HFM) as immune-isolation device, in order to increase islet longevity.

Following key questions that were raised in the present study:

1.Whether a systematic combination of bioactive molecules, acts as pancreatic islet differentiating agents from hBMSCs to functional ILCCs?

2. Which microRNAs play an important role in islet differentiation from hBMSCs?

3. Whether inhibiting candidate microRNAs accelerate initiation of islet differentiation process from hBMSCs?

4. Does bioactive molecules enhance the viability of encapsulated rat islets / ILCCs?

5.Whether HFM encapsulation protocol, increases islet longevity in "Rat to Mouse" xenotransplantation model?

Based on the aim and the rationale for the present study, we proposed the following objectives for the thesis:

2.1. Specific objectives:

1. Systematic evaluation of the combinatorial activity of bioactive molecules for islet neogenesis of human BMSCs into functional islets.
2. Assessment of molecular mechanism in islet differentiation pathway from hBMSCs using the best combination of bioactive molecules.
3. Assessment of microRNAs profile & microRNA modulation by silencing /inhibition (LNA) for augmentation of islet differentiation from hBMSCs.
4. Encapsulation & transplantation of islets into a diabetic mouse model for effective therapy.

The above-mentioned objectives are converted into four major chapters of this Ph.D. thesis

Chapter: 3 Systematic evaluation of the combinatorial activity of bioactive molecules for islet neogenesis of human BMSCs into functional islets.

(a) hBMSCs isolation and characterization
(b) hBMSCs differentiation into ILCCs (18^{th} Day protocol)
(c) Functional characterization of ILCCs

Chapter: 4 Assessment of molecular mechanism in islet differentiation pathway from hBMSCs using the best combination of bioactive molecules.

(a) Temporal gene expression profile during islet differentiation
(b) Temporal protein expression profile during islet differentiation

Chapter: 5 Assessment of microRNA profile & microRNA modulation by silencing /inhibition (LNA) for augmentation of islet differentiation from hBMSCs.

(a) microRNAs expression profile during islet differentiation
(b) Identified candidate microRNAs expression during islet differentiation
(c) microRNA power inhibitor [Power Lock Nucleic Acid (LNA)] LNA-has-miR-124a study

Chapter: 6 Encapsulation & transplantation of islets into a diabetic mouse model for effective therapy.

(a) ILCCs encapsulation study (*In vitro* study)
(b) Rat islet encapsulation study (*In vitro* study)
(c) "Rat to Mouse" for xeno-islet transplantation study using HFM (*In vivo*)