ABSTRACT

Diabetes Mellitus (DM) describes a group of metabolic disorders characterized by chronic hyperglycemia because the body becomes insulin resistant or doesn't produce enough insulin. It can lead to life-threatening health complications such as retinopathy, nephropathy, neuropathy, macrovascular problems with a major impact on the patient's lives and well-being of people, families, and societies worldwide. International Diabetes Foundation (ID) estimates 463 million (age group: 20–79 years) diabetics globally in the year of 2019. These numbers are expected to increase to 578 million by 2030 and 700 million by 2045. In spite of the fact that there are various modern therapeutics for managing diabetes mellitus by different types of insulin injectables as well as oral hypoglycemia medication, the everlasting cure for diabetes is still under development phase. Regenerative medicine and tissue engineering possessed much trust for the advanced treatment of diabetes mellitus. Thus, current research now focused on pancreatic islet differentiation applying stem cell technology. Among different adult stem cell sources, human bone marrow-derived mesenchymal stem cells (hBMSCs) have generated a great deal of interest in many clinical benchmarks due to the most clinically potent and ethically acceptable for the generation of functional islet-like cells clusters (ILCC). Thus, the aim of this thesis is to explore the differentiation potential of the hBMSCs to islets and improve the quality and quantity of the transplantable islets by bioactive molecules, miRNA approach, and islet encapsulation strategy.

hBMSCs were successfully isolated, expanded, and characterized by surface markers and trilineage differentiation. Our first objective was to develop novel and scalable islet differentiation protocol, in order to generate bona fide pancreatic islets from adult bone marrow-derived mesenchymal stem cells using herbal bioactive molecules for effective islet neogenesis. However, islet-like cell clusters previously generated from hBMSCs lack countless functional characteristics of pancreatic islets. In the current investigation, we report optimizing a multi-stage pancreatic islet differentiation protocol that can produce numerous of glucose-responsive pancreatic islets from hBMSCs *in vitro* utilizing bioactive molecule cocktail keeping activin A as a positive control. Further, we dissected the hBMSCs' differentiation route into pancreatic islets in a systematic stepwise process which recapitulated pancreatic islet development by directing cells through the stages resembling definitive endoderm, pancreatic progenitor, endocrine progenitor, and finally cell clusters that expressed pancreatic endocrine hormones.

To understand the mechanism of islet differentiation from hBMSCs, we performed the temporal transcriptional and protein profiling of all vital factors involved in islet differentiation at a different time interval (0 Day,5 Day,10 Day,15 Day and 18 Day) and noted that bioactive molecules cocktail is better islet differentiating agent as compared to activin A. This investigation gives a profound perspective on human bone marrow-derived mesenchymal stem cells (hBMSCs) differentiation into pancreatic islet-cells and application of stem cell-based therapy in diabetes.

Transcription factors are under the regulatory control of miRNA, hence, the next most important objective of this thesis was to examine if microRNAs play a crucial role during islet differentiation and if yes, then manipulation of a candidate microRNA can lead to accelerate the islet differentiation process. Thus, we screened 28 reported potent microRNAs at different time intervals (0Day, 5Day, 10Day, 15Day, and 18Day) in the islet differentiation process from hBMSCs. The result demonstrated that microRNA - miR-124a plays an important role in islet differentiation and its inhibition during the early stage of islet differentiation by LNA technology leads to the acceleration of the islet differentiation process from hBMSCs. These confounding results increase our understanding in the field of microRNAs and islet biology for the treatment of diabetes.

Next, we wanted to formulate novel islet encapsulation methods (*In-vitro*) using a combination of bioactive molecules and established "Rat to Mouse" xeno-islet-transplantation model system (*In vivo*) using Hollow Fiber Membrane (HFM). Preliminary *in vitro* study on rat islet and hBMSCs derived ILCCs demonstrated that the exploitation of bioactive molecules during the islet encapsulation process increases islet longevity. Xeno-islet-transplantation was successfully performed using encapsulated rat islets inserted in Hollow Fibre Membrane (HFM) in STZ diabetic mice, which resulted in normoglycemia of diabetic mice up to three months without the use of immunosuppressant drugs.

In conclusion, we evaluated clinically relevant approaches for islet transplantation in combination with islet encapsulation, in order to find a synergic outcome that may translate pre-clinical to clinical studies. By combining our expertise across disciplines such as bioactive molecule-based stem cell therapy, LNA technology along with HFM technology, we can begin to address the multiple challenges that are involved in translating stem cell therapy along with the islet transplantation approach from the laboratory to the clinic.