
TABLE OF CONTENTS

Abstract 1

Chapter 1: Introduction & Review Literature

1. Introduction..... 3

1.1. Pancreas..... 3

1.2. Various cell types of endocrine nature..... 4

1.2.1. α -Cells..... 4

1.2.2. β -Cells..... 5

1.2.3. Delta-Cells 7

1.2.4. PP Cells..... 7

1.2.5. Epsilon Cells 7

1.3. Mouse Pancreatic development..... 8

1.3.1. Signaling pathways regulating early pancreas development 8

1.3.2. The formation & differentiation of exocrine pancreatic cell type: Acinar 8

1.3.3. Generation of ductal epithelium and bipotent cell formation 9

1.3.4. The formation of pancreatic endocrine progenitor cells 9

1.3.5. Formation of pancreatic islets of Langerhans 10

A. Pancreatic endocrine cells delamination 10

B. Pancreatic endocrine cell migration, clustering, and assembly 10

1.4. Human pancreas development 12

1.5. Transcriptional control of islet development in the pancreas 14

1.5.1. Generation of Definitive Endoderm..... 15

A. HNFs (Hepatocyte nuclear factors) family of transcription factors..... 15

B. SOX9/SOX17 15

C. GATA4/GATA6 15

1.5.2. Formation of Pancreatic Progenitors 16

D. A. PDX1..... 16

E. B. PTF1A/P48..... 16

1.5.3. Establishment of the Endocrine Progenitors..... 17

A. NGN3..... 17

1.5.4. Endocrine Lineages Specification.....	17
A. PAX/ARX Transcription Factors.....	17
B. NKX Transcription Factors.....	17
1.5.5. Maintenance of pancreatic β -Cell Identity.....	18
A. NEUROD/BETA2	18
B. MAFA.....	18
1.6. Diabetes Mellitus.....	19
1.6.1. Epidemiology.....	19
1.6.2. Diagnosis.....	19
1.7. Type 1 Diabetes.....	20
1.7.1. Idiopathic type 1 diabetes	21
1.7.2. Fulminant type 1 diabetes	21
1.7.3. Causes of T1D.....	22
A. Genetic	22
B. Environmental.....	23
C. Chemicals and Drugs	24
1.7.4. Symptoms	24
1.7.5. Type 1 Diabetes Management	24
A. Diabetic diet and lifestyle modification.....	24
B. Insulin	24
(I) Regular insulin	25
(II) Zinc and NPH insulins	25
1.8. Pancreatic transplantation	26
1.8.1. Artificial pancreas	27
1.8.2. Islet transplantation.....	27
1.8.3. Islet encapsulation.....	28
1.9. Regenerative medicine	28
1.9.1. Reprogramming of pancreatic β -cells and islet neogenesis.....	28
1.9.2. Stem Cell-Based Therapies.....	29
A. Human embryonic stem cells.....	30
B. Induced pluripotent stem cells	31
1.10. Human Bone Marrow Mesenchymal Stem Cells (hBMSCs)	32

1.11.	MicroRNAs (microRNAs).....	33
1.11.1.	Biogenesis of microRNAs.....	33
1.11.2.	Role of microRNA in the pancreas	35
1.11.3.	MicroRNA manipulation strategy:.....	37
A.	microRNA overexpression.....	38
B.	microRNA knockdown	38
1.12.	chemical agents promoting pancreatic islet differentiation	38
1.12.1.	Activin A and Betacellulin.....	39
1.12.2.	Exendin-4	40
1.12.3.	FGF, EGF, KGF, and HFG	41
1.12.4.	Retinoic acid (RA)	42
1.12.5.	Sodium Butyrate.....	43
1.12.6.	Nicotinamide	43
1.13.	Antidiabetic activity of isolated compounds from <i>Enicostemma littorale</i> :	43
1.13.1.	Swertisin as pancreatic islet neogenic agent:	44
1.14.	Islet differentiation from various sources of adult mesenchymal stem cells	46

Chapter 2 : Aims and Objectives

2.	Aim and Objectives.....	55
2.1.	Specific objectives.....	56

Chapter 3: Systematic evaluation of combinatorial activity of bio-active molecules for islet neogenesis of hBMSCs into functional islets.

3.1.	Introduction	58
3.2.	Experimental design.....	62
3.3.	Material and method.....	63
3.3.1.	Materials	63
3.3.2.	Methods	63
A.	Isolation and characterization of hBMSCs	63
B.	Cell count and growth curve	64
C.	Flow cytometric analysis of surface marker and intracellular marker staining	64
i.	Surface marker	64
ii.	Intracellular marker.....	65

D. Immunocytochemistry	65
E. Western blot analysis	66
F. Gene expression study	66
G. Differentiation of hBMSCs into adipocytes, osteocytes, chondrocyte (Trilineage Differentiation).....	67
H. Islets Differentiation protocol	67
I. DTZ staining protocol.....	68
J. MTT assay	68
K. FDA-PI staining	69
L. Annexin V-PI staining protocol for apoptosis assay.....	69
M. Intracellular reactive oxygen species (ROS) detection assay	69
i. Multimode reader	69
ii. Inverted fluorescence microscopy	70
N. Human c-peptide release assay	70
O. Statistical analysis.....	71
3.4. Results	71
3.4.1. hBMSCs isolation from healthy human bone marrow	71
3.4.2. Characterization of patient-derived hBMSCs.....	72
3.4.3. Gene and protein expression of stemness markers in isolated hBMSCs.....	73
A. Protein Expression (Western Blot)	74
B. Gene Expression (qPCR)	74
3.4.4. Trilineage differentiation potential of hBMSCs	75
A. Adipocyte differentiation	75
B. Osteocyte differentiation	76
C. Chondrocyte differentiation	76
3.4.5. Differentiation of hBMSCs into ILCCs (10 th Day protocol; Non-adherence plates)	77
3.4.6. Differentiation of hBMSCs into ILCCs (18 th Day protocol; non-adherence plates)	79
3.4.7. Differentiation of hBMSCs into ILCCs (18 th Day protocol; ultra-low adherence plates) * (Entire islet differentiation protocol is filed for Indian Patent)	82
3.4.8. Morphometry analysis of differentiated ILCCs.....	85

3.4.9. Cell viability assay 18 th day ILCCs	86
3.4.10. Measurement of cellular ROS in 18 th day differentiated ILCCs using DCFDA Assay	88
3.4.11. Gene expression of lineage-specific markers involved in islet differentiation of ILCCs	89
3.4.12. Islet functionality of 18 th Day ILCCs Immunocytochemistry.....	90
3.4.13. Flow cytometry analysis of pancreatic hormone expression in ILCCs.....	92
3.4.14. <i>In vitro</i> Human C-peptide release in response to a glucose challenge.....	93
3.5. Discussion	94
3.6. Summary of Chapter-3	100

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

4.1. Introduction	101
4.2. Experimental design of chapter-4	104
4.3. Plan of work Gene and Protein expression profile.....	105
4.4. Material & Methods	105
4.4.1. Materials	105
4.4.2. Islet differentiation from hBMSCs	105
4.4.3. Gene expression study	106
4.4.4. Immunocytochemistry study.....	106
4.4.5. Flow cytometry study	106
4.4.6. Western blot study	106
4.5. Results	106
4.5.1. Temporal gene expression during islet differentiation using Real-time qPCR ...	107
A. Definitive Endocrine genes	107
B. Pancreatic progenitor genes	109
C. Pancreatic Endocrine Progenitors	110
D. Functional pancreatic islets genes.....	111
4.5.2. Temporal protein expression during islet differentiation using Immunocytochemistry (ICC) assay	113
A. 5 th Day Cell Clusters	114
B. 10 th Day Cell Clusters	115

C. 15 th Day Cell Clusters	118
D. 18 th Day Cell Cluster	119
4.5.3. Immunocytochemistry assay confirms protein expression in single cells (Isolated from cell clusters)	122
4.5.4. Flow cytometry analysis support protein expression during islet differentiation	126
4.5.5. Temporal protein expression during islet differentiation using wester blotting..	128
4.6. Discussion	130
4.7. Summary of Chapter-4.....	136
 Chapter 5: Assessment of microRNA profile & microRNA modulation by silencing/inhibition (LNA)for augmentation of islet differentiation from hBMSCs.	
5.1. Introduction	137
5.1.1. miRNAs and Islet differentiation.....	137
5.1.2. microRNAs and Bioactive molecules.....	140
5.1.3. MicroRNA Manipulation strategies Inhibition (LNA)	140
5.2. Experimental design of chapter 5.....	143
5.3. Plan of work microRNAs profile	144
5.4. Material & Method.....	145
5.4.1. Materials	145
A. Chemicals and cell culture media	145
B. microRNAs kits	145
5.4.2. Methods	146
A. hBMSCs culture and <i>in vitro</i> islet differentiation protocol (18 th Day protocol)..	146
B. microRNAs isolation	146
C. microRNAs profile.....	146
D. microRNA Power Inhibitor LNA	148
E. Immunocytochemistry by Laser-scanner confocal microscopy	148
5.5. Results	149
5.5.1. microRNAs expression profile (<i>In vitro</i> differentiation of hBMSCs into ILCCs)	149

5.5.2. Dynamic changes in microRNAs expression Profile during multistage islet differentiation (5 th , 10 th , 15 th and 18 th day).....	152
5.5.3. microRNA 124a & its target FOXA2 gene and protein expression	154
5.5.4. microRNA power inhibitor (LNA-hsa-miR-124a) study	155
5.6. Discussion	157
5.7. Summary	163

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

6.1. Introduction	164
6.1.1. Regenerative Medicine	164
6.1.2. Islet transplantation	165
A. The strategy of islets transplantation	165
B. Limitations of islets transplantation.....	165
6.1.3. Islets Encapsulation	166
A. Types of encapsulation	167
B. Challenges in islets encapsulation	168
6.1.4. Encapsulation materials and its characteristics.....	168
6.1.5. Hollow Fibre Membrane (HFM)	170
6.1.6. Bio-active Molecules	171
6.2. Experimental design for chapter-6	173
6.3. Material and Method	175
6.3.1. Material	175
A. Chemicals, cell culture media, and HFM.....	175
B. Animal (Mice & Rats)	175
6.3.2. hBMSCs differentiation into ILCCs using Bioactive molecule cocktails	176
6.3.3. Rat islets isolation.....	176
6.3.4. DTZ Staining	176
6.3.5. Islets Encapsulation	176
6.3.6. Annexin V/PI Staining	177
6.3.7. Xeno-islets transplantation study	177
6.3.8. H & E staining	178
6.3.9. Statistical analysis	178

6.4. Results	178
6.4.1. Encapsulated ILCCs viability <i>In vitro</i> study.....	178
6.4.2. Encapsulated rat islet viability <i>In vitro</i> study	181
6.4.3. Encapsulated Rat Islets transplantation (HFM) in STZ treated diabetic mice (Xenotransplantation- in-vivo study).....	183
6.4.4. Retrieval of xenotransplanted rat islets from HFM	185
6.5. Discussion	187
6.6. Summary of Chapter-6.....	193
 Chapter 7: Summary and conclusions	
7.1. Summary	194
7.2. Overall conclusions.....	197
7.3. Thesis summary.....	199
8. References	200
 I. Appendix	
II. List of Tables and Figures	
III. Publications and Patent	
IV. Conferences	
V. Synopsis	