

**GENE THERAPY FOR DIABETES: LENTIVIRAL
EXPRESSION OF INSULIN IN LIVER CELLS**

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**MASTER OF SCIENCE
(BY RESEARCH)**

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CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Prudence Nicol Gatt

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All I need is a little sign,
To get behind this sun and cast this weight of mine,
All I need is the place to find,
And there I'll celebrate.

~All I Need; Air

PUBLICATIONS AND PRESENTATIONS

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TABLE OF CONTENTS

GENE THERAPY FOR DIABETES: LENTIVIRAL EXPRESSION OF INSULIN IN LIVER CELLS.....	i
CERTIFICATE OF AUTHORSHIP.....	ii
ACKNOWLEDGEMENTS.....	iii
PUBLICATIONS AND PRESENTATIONS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xii
LIST OF TABLES.....	xiv
ABBREVIATIONS.....	xv
ABSTRACT.....	xvii
CHAPTER ONE.....	1
Introduction.....	1
1.1 Definition.....	2
1.2 Classification of diabetes mellitus.....	2
1.2.1 Type 2 Diabetes.....	2
1.2.2 Type 1 Diabetes.....	3
1.3 Pathogenesis of T1D.....	5
1.4 Complications.....	9
1.5 Treatments for T1D.....	10
1.5.1 Insulin therapy.....	10
1.5.2 Transplantation therapy.....	11
1.5.2.1 Whole pancreas transplantation.....	11
1.5.2.2 Islet cell transplantation.....	12
1.5.2.3 Problems associated with transplantation therapies.....	13
1.5.3 Stem cells.....	13
1.5.4 Gene therapy.....	14
1.5.4.1 Methods of in vivo gene therapy delivery and their applications.....	15
1.6 Gene therapy for type 1 diabetes.....	21

1.6.1	β -cell differentiation, insulin synthesis, storage and secretion.....	21
1.6.2	Target cell types previously used in gene therapy of type 1 diabetes	27
1.6.3	Use of promoters and enhancers to direct the viral vector transgene expression.....	31
1.7	Applications of HMD and HMD/INS-FUR lentiviral vectors for gene therapy.....	32
1.7.1	Structure of HMD and HMD/INS-FUR lentiviral constructs	32
1.7.2	Delivery of HMD and HMD/INS-FUR to the livers of STZ-induced diabetic Wister rats	34
1.7.3	Reversal of autoimmune T1D by the delivery of HMD and HMD/INS-FUR to the livers of NOD mice	38
1.7.4	Comparison of HMD/INS-FUR and WPT/INS-FUR lentiviral vector constructs	41
1.8	Aims of project.....	43
CHAPTER TWO		44
Materials and Methods.....		44
2.1	Materials.....	45
2.1.1	General materials and reagents	45
2.1.2	Transformation and plasmid isolation.....	45
2.1.3	Calcium phosphate transfection of viral vectors	46
2.1.4	Viral vector purification by Tangential Flow Filtration (TFF)	46
2.1.5	Determination of viral titre by flow cytometric analysis	46
2.1.6	Standard and quantitative RT-PCR amplification.....	47
2.1.7	WPT viral vector transfection	47
2.1.8	Fluorescence activated cell sorting (FACS).....	47
2.1.9	Radioimmunoassay (RIA).....	47
2.1.10	Detection of insulin in viral vector transduced cell lines	48
2.2	Methods.....	49
2.2.1	Sterility and containment	49
2.2.2	Transformation of plasmids Δ R8.2, VSV-G, HMD or HMD/INS-FUR, plasmid isolation and restriction digest.....	49

2.2.2.1	Transformation of plasmids Δ R8.2, VSV-G, HMD or HMD/INS-FUR ..	49
2.2.2.2	Small scale plasmid isolation	50
2.2.2.3	Restriction digestion of isolated plasmids and long-term storage.....	51
2.2.2.4	Large scale plasmid isolation	51
2.2.3	Quantification and analysis of nucleic acid samples.....	53
2.2.3.1	Nucleic acid quantification.....	53
2.2.3.2	Agarose gel electrophoresis.....	53
2.2.4	Calcium phosphate transfection of HMD and HMD/INS-FUR viral vectors ..	54
2.2.5	Viral vector purification by Tangential Flow Filtration (TFF)	55
2.2.5.1	Assembly of TFF unit for the use in TFF system	55
2.2.5.2	Preparation of a new membrane cassette for the use in TFF machine .	57
2.2.5.3	Preparation of the membrane cassette for viral vector concentration ...	59
2.2.5.4	Viral vector purification and concentration using TFF machine	60
2.2.5.5	Cleaning of the TFF apparatus	61
2.2.6	Viral vector titre determination	62
2.2.6.1	Traditional transduction method for viral vector titre determination....	62
2.2.6.2	Optimisation of transduction parameters for viral vector titre determination	62
2.2.7	Determination of viral titre by flow cytometric analysis	64
2.2.7.1	Acquiring data of transduced cells with viral vector by flow cytometry	64
2.2.7.2	Analysis of flow cytometric data of transduced cells with viral vector	65
2.2.8	RNA isolation.....	66
2.2.8.1	RNeasy kit.....	66
2.2.8.2	TRIzol method.....	68
2.2.9	DNase I digest treatment of isolated RNA for RT-PCR	69
2.2.10	Synthesis of cDNA.....	69
2.2.11	RT-PCR amplification of transcription factors	70

2.2.12	Quantitative RT-PCR amplification of transcription factor PDX-1 in HMD and HMD/INS-FUR treated NOD mice liver samples	72
2.2.12.1	DNase I digest treatment of isolated RNA for quantitative RT-PCR .	72
2.2.12.2	Synthesis of cDNA for qRT-PCR	72
2.2.12.3	qRT-PCR primer matrix of transcription factor PDX-1 primer sets and housekeeping genes β 2M and HPRT	73
2.2.12.4	qRT-PCR of transcription factor PDX-1 comparing HMD and HMD/INS-FUR viral vector transduced NOD mouse liver samples...	74
2.2.13	Transformation of plasmids pWPT or pWPT/INS-FUR, plasmid isolation and restriction digest	75
2.2.14	Optimisation of calcium phosphate transfection of WPT/INS-FUR plasmid	75
2.2.15	Calcium phosphate transfection of pWPT and pWPT/INS-FUR viral vectors	77
2.2.16	Optimisation of transduction using Huh7 liver cell line with viral vectors	77
2.2.17	FACS of viral vector transduced Huh7 liver cell line	78
2.2.18	Radioimmunoassay	81
2.2.19	Detection of insulin from FACS sorted cell lines	83
2.2.19.1	Insulin secretion	83
2.2.19.2	Insulin storage	84
2.2.19.3	Insulin stimulation.....	84
2.2.19.4	Insulin stimulation visualised by microscope detection.....	85
CHAPTER THREE.....		87
Viral vector transfection, concentration and titre determination		87
3.1	Introduction	88
3.2.1	Plasmid Δ R8.2, VSV-G, HMD or HMD/INS-FUR transformation, isolation and restriction enzyme digestion.....	89
3.2.2	Optimisation of transduction parameters for viral vector titre determination, following the transfection and concentration of HMD and HMD/INS-FUR viral vectors	91

3.3	Discussion	100
CHAPTER FOUR.....		104
Expression of pancreatic markers in liver tissue after reversal of autoimmune diabetes in NOD mice		104
4.1	Introduction	105
4.2.1	Optimisation of RNA isolation of murine tissue for expression studies....	106
4.2.2	Expression pancreatic transcription factors and other genes in INS-FUR transduced liver and pancreas	113
4.2.3	Quantitative RT-PCR and Western blot analysis of <i>Pdx-1</i> in HMD and HMD/INS-FUR viral vector transduced NOD mouse liver samples.....	115
4.3	Discussion	119
CHAPTER FIVE.....		125
Comparison of HMD and WPT lentiviral vector promoters.....		125
5.1	Introduction	126
5.2.1	Plasmids WPT and WPT/INS-FUR transformation, isolation and restriction enzyme digest.....	127
5.2.2	Optimisation of calcium phosphate transfection of 293T cells with the WPT/INS-FUR plasmid and titre of WPT and WPT/INS-FUR viral vectors .	129
5.2.3	Viral vector transduction of Huh7 liver cell and fluorescence activated cell sorting of transduced cells.....	133
5.2.4	Quantification of secreted and stored insulin in flow cytometric sorted viral vector transduced cell populations	137
5.2.5	Glucose-stimulated insulin secretion by HI cell populations of the HMD/INS-FUR-75 and WPT/INS-FUR-50 viral vectors	141
5.2.6	Confocal microscopic analysis of stored insulin within the HI cell population transduced with the HMD/INS-FUR-75 viral vector.....	143
5.3	Discussion	146

CHAPTER SIX	155
Conclusion and future direction	155
6.1 Conclusion.....	156
6.2 Future direction	158
References	160

LIST OF FIGURES

Figure 1.1: The stages in the development of T1D.....	5
Figure 1.2: Recognition-linked mechanism of islet β -cell death	7
Figure 1.3: Activation-linked mechanism of islet β -cell death.....	8
Figure 1.4: Endogenous insulin secretion and inhibition.....	11
Figure 1.5: Schematic diagram of the various pathways to differentiate cell lineage determination.....	15
Figure 1.6: Schematic drawing of the Structure of HIV-1 lentiviral vector genome.....	20
Figure 1.7: Linear cascade of pancreatic β -cell transcription factors	22
Figure 1.8: Process of pre-proinsulin cleavage into mature insulin.....	23
Figure 1.9: Secretion of insulin from a pancreatic β -cell.....	25
Figure 1.10: Schematic drawing of furin-cleavable proinsulin with B-10 mutation	26
Figure 1.11: Schematic drawing of the HMD and HMD/INS-FUR vector constructs.....	33
Figure 1.12: Blood glucose concentrations of HMD and HMD/INS-FUR treated Wistar rats	35
Figure 1.13: Expression of β -cell transcription factors and pancreatic hormones in viral vector transduced Wistar rat livers.....	37
Figure 1.14: Blood glucose levels of diabetic NOD mice treated with HMD and HMD/INS- FUR viral vectors	39
Figure 1.15: Expression of insulin following reversal of spontaneous type 1 diabetes in HMD/INS-FUR treated NOD mice	40
Figure 1.16: Schematic drawing of the pWPT and pWPT/INS-FUR vector constructs.....	42
Figure 2.1: Tangential Flow Filtration unit.....	56
Figure 2.2: Schematic of TFF system	58
Figure 2.3: Analysis of flow cytometric plots to determine viral vector titre.....	66
Figure 2.4: FACS of viral vector transduced Huh7 liver cell line	80
Figure 3.1: Agarose gel electrophoresis of restriction enzyme digested plasmids	90
Figure 3.2: Determination of viral titre by microscopy	92
Figure 3.3: Flow cytometric fluorescence detection of viral vector transduced cell line	93
Figure 3.4: Flow cytometric analysis for viral vector titre optimization method 1a.....	96

Figure 3.5: Flow cytometric analysis for viral vector titre optimization method 1b	97
Figure 3.6: Flow cytometric analysis for viral vector titre optimization method 1c.....	98
Figure 3.7: Flow cytometric analysis for HMD viral vector titre	99
Figure 4.1: Isolation of RNA from NOD mouse liver and pancreatic samples using RNeasy kit methodology	110
Figure 4.2: Isolation of RNA from NOD mouse liver and pancreatic tissue using TRIzol reagent.....	112
Figure 4.3: RT-PCR amplification of pancreatic transcription factors	114
Figure 4.4: Relative quantification of <i>Pdx-1</i> expression after HMD/INS-FUR transduced NOD mice liver tissue.....	116
Figure 4.5: Relative Quantitative of <i>Pdx-1</i> expression after HMD transduced NOD mice liver tissue	117
Figure 4.6: Comparison of transcription factor Pdx-1 expression from transduced liver tissue samples by real-time RT-PCR quantification	118
Figure 4.7: Western blot analysis for <i>Pdx-1</i> protein.	118
Figure 5.1: Agarose gel electrophoresis of restriction enzyme digested plasmids	128
Figure 5.2: Optimisation of WPT/INS-FUR plasmid transfection	130
Figure 5.3: Flow cytometric analysis of viral vector titre.....	132
Figure 5.4: Flow cytometry analysis of HMD/INS-FUR viral vector transduced Huh7 cells	135
Figure 5.5: Flow cytometry analysis of WPT and WPT/INS-FUR viral vector transduced Huh 7 cells	136
Figure 5.6: Secretion of insulin from viral vector transduced cell lines	138
Figure 5.7: Insulin storage of viral vector transduced Huh7 cell populations	140
Figure 5.8: Glucose-stimulated insulin secretion of viral vector transduced Huh7 cells ..	142
Figure 5.9: Confocal microscopy of the HI cell line of HMD/INS-FUR-75 viral vector..	144
Figure 5.10: Fluorescent intensity of stored insulin.....	145

LIST OF TABLES

Table 1.1: Etiological classification of diabetes mellitus and related abnormal glucose metabolism	4
Table 1.2: Summary of target cell types for gene therapy of T1D	30
Table 2.1: Primer sequences used to determine expression levels of genes of interest	71
Table 2.2: qRT-PCR primers used to determine expression of pancreatic β -cell transcription factor <i>Pdx-1</i>	73
Table 2.3: qRT-PCR Primer matrix used to determine the optimal primer concentration for qRT-PCR analyses	74
Table 2.4: pWPT/INS-FUR concentrations at various time points used to determine the optimal parameters for pWPT/INS-FUR transfection	76
Table 2.5: Amounts of concentrated viral vector used to transduce Huh7 liver cell line to determine optimal transducing units of viral vector per cell.....	78
Table 2.6: Contents of RIA tubes.....	82
Table 3.1: Yield and purity of plasmids following transformation.....	89
Table 3.2: Viral vector titre and fold difference between two titre determination methods	95
Table 4.1: Yield, purity and integrity of mouse RNA following extraction protocols, values given in mean \pm SEM.....	108
Table 5.1: Yield and purity of plasmids following transformation.....	127

ABBREVIATIONS

α	alpha
β	beta
β 2M	beta two microglobulin
δ	delta
γ	gamma
$^{\circ}$ C	degrees celsius
Ψ	packaging signal
ABS	Australian Bureau of Statistics
AIHW	Australian Institute of Health of Welfare
APC	antigen-presenting cell
CD4 ⁺	T-cell co-receptor
CD8 ⁺	T-cell co-receptor
cDNA	complementary deoxyribonucleic acid
CFTR	cystic fibrosis transmembrane conductance regulator
DMEM	dulbecco's modified eagle medium
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DOPE	1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine
DOTAP	1, 2-dioleoyl-sn-glycero-3-trimethylammonium-propane
EDTA	ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescent protein
EF1- α	elongation factor 1-alpha
ESRD	end-stage renal disease
FBS	foetal bovine serum
FFO	intervallic infusion in full flow occlusion
h	hour
H ₂ O	water
HEPES	N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMD	HIV/murine stem cell virus promoter/enhancer hybrid
HPRT	Hypoxanthinephosphoribosyltransferase
IDF	International Diabetes Federation
IFN- γ	interferon gamma
IL	interleukin
INS-FUR	furin cleavable human proinsulin
IVGTT	intravenous glucose tolerance test
LTR	long term repeat
M	molarity
MHC	major histocompatibility complex
min	minute
MOI	multiplicity of infection (a ratio of viral infectious particles per cell)
MSCV	murine stem cell virus

N ₂ (l)	liquid nitrogen
NOD	non-obese diabetic
NaOCl	sodium hypochlorite
NaOH	sodium hydroxide
O/N	overnight
PCR	polymerase chain reaction
ppm	parts per million
PS	penicillin and streptomycin
pWPT	elongation factor 1-alpha/woodchuck hepatitis virus posttranscriptional regulatory element promoter/enhancer
q	quantitative
RIA	radioimmunoassay
RNA	ribonucleic acid
RNase	ribonuclease
RT	room temperature
s	second
SCID	severe combined immunodeficiency
STZ	streptozotocin
T-cell	T-lymphocyte (white blood cell)
TFF	tangential flow filtration
T1D	Type 1 diabetes mellitus
TNF- α	Tumour Necrosis Factor alpha
T _M	melting temperature
U	units
μ	micro
μ L	microlitre
v/v	volume/volume
WHO	World Health Organisation
WPRE	woodchuck hepatitis virus posttranscriptional regulatory element
w/v	weight/volume

ABSTRACT

Type 1 diabetes mellitus (T1D) is caused by the autoimmune destruction of the insulin producing pancreatic beta (β)-cells. Current treatment is by multiple daily insulin injections, which cannot mimic the minute-to-minute responsiveness of β -cells *in vivo*, leading to the development of chronic complications, which increase morbidity and mortality. This study investigated the use of lentiviral vectors to deliver the furin-cleavable proinsulin gene (INS-FUR) to liver cells with the goal of generating glucose-responsive, insulin secreting surrogate β -cells.

A methodology for viral titre determination using flow cytometry was developed, optimised and validated. This protocol was compared to the use of fluorescence microscopy for titre determination. The latter underestimated the viral titre as accurate quantification of transduced cells (enhanced green fluorescent protein (EGFP)-positive) was not possible due to the inability to count individual cells, which were in confluent monolayer clusters. Furthermore, cells transduced using a low multiplicity of infection (MOI; a ratio of viral infectious particles per cell) were indistinguishable from background fluorescence, while high MOI significantly underestimated the true functional viral titre. Flow cytometry enabled the determination of single or low MOI events and thus increased the accuracy of the viral titre determination.

The second aim of the study was to examine liver tissue from spontaneous diabetic non-obese diabetic (NOD) mice, which had normalised their blood glucose following the delivery of furin-cleavable insulin in the HIV/murine stem cell virus promoter/enhancer hybrid (HMD) lentiviral vector (HMD/INS-FUR), for evidence of pancreatic transcription factors and hormones, indicative of liver to pancreas transdifferentiation. The HMD/INS-FUR-treated NOD mice normalised blood glucose levels 24 h after viral vector delivery and normoglycaemia was maintained for 150 days (experimental end point). The transduced liver tissue showed the presence of insulin storage granules and the expression of several pancreatic transcription factors and hormones, including *Pdx-1*, *Neurod1*, *Ngng3*, *Pax 4*, *Nkx2.2*, glucagon and somatostatin. Furthermore, the induction of mouse insulin 1

expression was detected and this phenomenon may be attributable to the expression of *MafA* and *MafB* transcription factors, which are known to play crucial roles in insulin expression late in the hierarchy of β -cell maturation. These results indicated that the delivery HMD/INS-FUR lentiviral vector to liver tissue induces hepatocyte to β -cell transdifferentiation and therefore holds therapeutic potential for the reversal of autoimmune T1D.

The final aim of this study investigated lentiviral vector construct design, and compared the HMD/INS-FUR vector construct with the newly acquired WPT/INS-FUR lentiviral construct, which possessed the elongation factor 1-alpha promoter with the woodchuck hepatitis virus post-transcriptional regulatory element enhancer (WPT). The ability of each lentiviral vector to transduce the Huh7 liver cell line using various MOI, insulin secretion, storage and glucose-regulated secretion were compared. Huh7 cells were readily transduced with the HMD/INS-FUR vector at an MOI of 75, however attempts at transducing the cells with the WPT/INS-FUR vector at an MOI >50, resulted in inhibition of cell growth. This was not the case with the empty-WPT vector, which implies the INS-FUR gene expressed in this vector putatively inhibited metabolic cell function at the higher MOI of 75. Insulin secretion was the same irrespective of the MOI used to transduce the Huh7 cells. By comparison, the WPT/INS-FUR viral vector-transduced cells stored a significantly higher concentration of insulin when compared to cells transduced with the 50 and 75 MOI of the HMD/INS-FUR viral vector ($p < 0.0001$ and $p = 0.001$, respectively). Therefore, fewer viral particles of the WPT/INS-FUR virus were required to achieve similar insulin secretion concentrations as the HMD/INS-FUR virus, while also achieving a higher concentration of insulin storage. However, as the total level of insulin stored after transfection with the WPT/INS-FUR viral vector was low (0.386 ± 0.041 pmoles/ 10^6 cells), these results must be viewed with caution.

Overall, this study has optimised an appropriate methodology for the accurate determination of viral titre for downstream applications. Furthermore, the study demonstrated the NOD mice transduced with the HMD/INS-FUR viral vector have the ability to store and secrete insulin in a glucose-regulated manner, through the induction of

pancreatic β -cell transcription factors and hormones. Moreover, the WPT/INS-FUR viral vector was found to be comparable to HMD/INS-FUR viral vector with respect to insulin secretion. However, as the former stored significantly more insulin *in vitro*, this suggests that the WPT/INS-FUR viral vector may be a useful construct for future *in vivo* studies investigating the reversal of T1D in NOD mice via hepatocyte transduction.