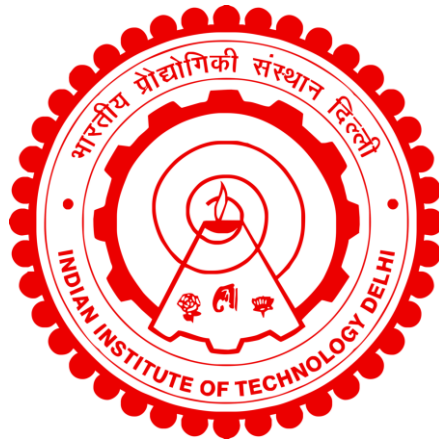


**DEVELOPMENT OF BIO-INSPIRED FORMULATIONS
TO MODULATE PANCREATIC MICROENVIRONMENT
FOR THE AMELIORATION OF TYPE I AND TYPE
II DIABETES**

ANJALI SINGH



**CENTRE FOR BIOMEDICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI
NOVEMBER 2024**

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Development of bio-inspired formulations to modulate pancreatic microenvironment for the amelioration of type I and type II diabetes

by

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CENTRE FOR BIOMEDICAL ENGINEERING

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

November 2024

**Dedicated
To
My Family**

Declaration

I hereby declare that the thesis entitled “**Development of bio-inspired formulations to modulate pancreatic microenvironment for the amelioration of type I and type II diabetes**” submitted to the Centre for Biomedical Engineering, IIT Delhi, is an original research work conducted by me under the supervision of Dr. Jayanta Bhattacharyya and is submitted for the award of the degree of philosophy in Biomedical engineering. The findings of this thesis have not been submitted elsewhere for the award of any degree/diploma.

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Acknowledgements

Completing this Ph.D. thesis has been a challenging yet immensely rewarding journey, and I am deeply grateful to everyone who has contributed to its realization. First and foremost, I would like to express my deepest gratitude to my supervisor, Prof. Jayanta Bhattacharyya, for his invaluable guidance, constant encouragement, and immense patience throughout this process. Their insightful feedback and unwavering support have been instrumental in the completion of this thesis. I am also grateful to my committee members, Prof. Prashant Mishra, Prof. Bishwajit Kundu, and Prof. Sandeep Kumar Jha, for their time, effort, and insightful comments that greatly improved the quality of my research. A heartfelt thank you goes to my labmates and friends; Anjali, Ahana, Vidit, Monu, Anindita, Shabnam, Abhishek, Palak, Ramya, Akshi, Zainab. Their camaraderie, stimulating discussions, and mutual support made this journey enjoyable and enriching. I am also indebted to Prof. Subhrata Kumar Pore and Animal facility at Amity University for providing me with the necessary resources and a conducive environment for research. The administrative staff have been incredibly helpful and efficient. I am deeply thankful to CSIR for providing my Ph.D. fellowship and enabled me to pursue this research and focus wholeheartedly on my academic endeavors.

On a personal note, I would like to extend my heartfelt gratitude to my parents, my brother, and my husband, for their unwavering support and encouragement throughout this journey. To my parents, thank you for instilling in me the values of hard work and perseverance. Your continuous love, guidance, and sacrifices have provided me with the foundation and strength to pursue my dreams. I am forever grateful for the countless ways you have supported me, both emotionally and practically, during the course of my studies. To my husband, Navdeep Singh, your patience and understanding during the late nights and long hours I spent on this thesis were more than I could have ever asked for. Thank you for always believing in me, even when

I doubted myself, and for being my rock throughout this process. Your love and sacrifices have made this accomplishment possible, and I am endlessly grateful for your constant presence by my side. This thesis is as much yours as it is mine.

Lastly, I would like to acknowledge all the unnamed individuals who have indirectly contributed to my research and personal growth during this period. Their influence and support, no matter how small, have been deeply appreciated.

Thank you all.

Anjali Singh

Abstract

Diabetes mellitus (DM), is a metabolic condition with persistent low grade chronic inflammation and is commonly diagnosed by high blood glucose level. In type 1 DM (T1DM), the loss of insulin synthesizing pancreatic β cell is responsible for hyperglycemia. However, decreased insulin secretion and higher insulin resistance is associated with type 2 DM (T2DM). This insulin insufficiency leads to hyperglycemia induced oxidative stress, chronic inflammation, macrophage infiltration in the pancreas, eventually damaging pancreatic β cells. Additionally, metabolic imbalance, hyperglycemia, and chronic inflammation leads to deposition of free fatty acids in hepatocytes causing hepatotoxicity. Clinically, exogenous insulin is used for the management of T1DM while, for T2DM, metformin is the first line of treatment. The other commonly prescribed treatments for T2DM includes sulphonylureas, meglitinides, sodium-glucose transport protein-2 inhibitors, incretin analogues, and insulin. Moreover, the combination of drugs with complementary mechanism of action provides better anti-diabetic efficacy when used clinically. Though, these treatments can help to regulate blood glucose level, still, these treatment regimens are associated with increased incidences of hypoglycemia, diabetic ketoacidosis, increased body weight, urinary tract infections, short plasma half-life, gain of insulin resistance, and loss of pancreatic β cells over time. Further, most of the clinically available therapies aimed to regulate blood glucose and do not improve insulin resistance and pancreatic β functions, hence, the underlying pathophysiology remains unresolved and fails to provide a complete solution.

Previous studies showed that the expression of the angiotensin II type 1 receptor (AT1R) is higher in diabetic conditions and its activation increases oxidative stress, damages endoplasmic reticulum, and leads to pancreatic β cell apoptosis. Moreover, telmisartan (TEL) which is an AT1R blocker (ARB) can reduce insulin resistance, restore oxidative balance, decrease ROS

levels, increase insulin secretion, and improve the morphology of pancreatic islet. However, like other small molecule drugs, TEL is associated with drawbacks including short plasma half-life, non-specificity, poor *in vivo* efficacy, require frequent and high dosage to achieve therapeutic benefits, causing dose-dependent toxicities to the healthy organs.

In literature, encapsulation of drugs inside nanoparticle-based delivery systems have shown a great efficacy to overcome the limitation of these small molecule drugs. To this end, this thesis is aimed to develop TEL nanoformulations using MIN6-derived extracellular vesicles (CEV) which not only act delivery vehicle but impart therapeutic benefit. Moreover, to show that CEV impart therapeutic benefit and compare the efficacy of TEL loaded CEV, we have synthesized lipoTEL by encapsulating TEL in to biomimetic lipid nanoparticles (BLN) mimicking lipid compositions of CEV. Our study showed that treatment of murine T1DM mice with nanoTEL outperforms lipoTEL by restoring the function of pancreatic β cells through the modulation of pancreatic inflammatory microenvironment. However, treatment with nanoTEL failed to reduce blood glucose levels over time. Further, T2DM which constitutes ~90% of total diabetes cases, has multidimensional complications. Thus, to address complication imparted by the hyperglycemic microenvironment of T2DM, a glucose-responsive bio-inspired formulation, termed Diabogel, containing modified glucagon like peptide-1, TEL, and CEV was developed. *In vitro* and *in vivo* efficacy analysis showed, treatment with Diabogel can address multifaceted complication of T2DM resulting into reduced blood glucose, improved pancreatic β cell functions while simultaneously reducing hepatotoxicity.

सार

मधुमेह मेलिटस (डीएम), लगातार निम्न श्रेणी की पुरानी सूजन के साथ एक चयापचय स्थिति है और आमतौर पर उच्च रक्त शर्करा स्तर से इसका निदान किया जाता है। टाइप 1 डीएम (टी1डीएम) में, इंसुलिन संश्लेषण करने वाली अग्राशयी β कोशिका के नुकसान के लिए रक्त शर्करा स्तर जिम्मेदार है। हालाँकि, इंसुलिन स्राव में कमी और उच्च इंसुलिन प्रतिरोध टाइप 2 डीएम (टी2डीएम) से जुड़ा है। इस इंसुलिन की कमी से उच्च रक्त शर्करा प्रेरित ऑक्सीडेटिव तनाव, पुरानी सूजन, अग्राशय में मैक्रोफेज की घुसपैठ होती है, जो अंततः अग्राशय β कोशिकाओं को नुकसान पहुंचाती है। इसके अतिरिक्त, चयापचय असंतुलन, उच्च रक्त शर्करा और पुरानी सूजन से हेपेटोसाइट्स में मुक्त फैटी एसिड का जमाव होता है, जिससे हेपेटोस्टैटोसिसिटी होती है। चिकित्सकीय रूप से, T1DM के प्रबंधन के लिए बहिर्जात इंसुलिन का उपयोग किया जाता है, जबकि T2DM के लिए, मेटफॉर्मिन उपचार का पहला तरीका है। टी2डीएम के लिए आमतौर पर निर्धारित अन्य उपचारों में सल्फोनीलुरिया, मेगालिटिनाइड्स, सोडियम-ग्लूकोज ट्रांसपोर्ट प्रोटीन-2 अवरोधक, इन्क्रेटिन एनालॉग्स और इंसुलिन शामिल हैं। इसके अलावा, चिकित्सीय रूप से उपयोग किए जाने पर पूरक क्रियाविधि वाली दवाओं का संयोजन बेहतर मधुमेह विरोधी प्रभावकारिता प्रदान करता है। हालाँकि, ये उपचार रक्त शर्करा के स्तर को नियंत्रित करने में मदद कर सकते हैं, फिर भी, ये उपचार निम्न रक्त शर्करा स्तर, मधुमेह केटोएसिडोसिस, शरीर के वजन में वृद्धि, मूत्र पथ के संक्रमण, अल्प प्लाज्मा आधा जीवन, इंसुलिन प्रतिरोध में वृद्धि और समय के साथ अग्राशय β कोशिकाओं की हानि की बढ़ती घटनाओं से जुड़े हैं। इसके अलावा, अधिकांश चिकित्सीय रूप से उपलब्ध उपचारों का उद्देश्य रक्त शर्करा को नियंत्रित करना है और इंसुलिन प्रतिरोध और अग्राशयी β कार्यों में सुधार नहीं करना है, इसलिए, अंतर्निहित पैथोफिज़ियोलॉजी हल नहीं होती है और पूर्ण समाधान प्रदान करने में विफल रहती है।

पिछले अध्ययनों से पता चला है कि एंजियोटेंसिन II टाइप 1 रिसेप्टर (AT1R) की अभिव्यक्ति मधुमेह की स्थिति में अधिक होती है और इसकी सक्रियता ऑक्सीडेटिव तनाव को बढ़ाती है, एंडोप्लाज्मिक रेटिकुलम को नुकसान पहुंचाती है, और अग्राशयी β सेल एपोप्टोसिस की ओर ले जाती है। इसके अलावा, टेलिमिसर्टन (TEL) जो एक AT1R अवरोधक (ARB) है, इंसुलिन प्रतिरोध को कम कर सकता है, ऑक्सीडेटिव संतुलन को बहाल कर सकता है, प्रतिक्रियाशील ऑक्सीजन प्रजातियों के स्तर को कम कर सकता है, इंसुलिन साव को बढ़ा सकता है और अग्राशयी आइलेट की आकृति विज्ञान में सुधार कर सकता है। हालांकि, अन्य छोटी अणु दवाओं की तरह, TEL कम प्लाज्मा आधा जीवन, गैर-विशिष्टता, विवो प्रभावकारिता में खराब, चिकित्सीय लाभ प्राप्त करने के लिए लगातार और उच्च खुराक की आवश्यकता सहित कमियों से जुड़ा हुआ है, जिससे स्वस्थ अंगों में खुराक पर निर्भर विषाक्तता होती है।

साहित्य में, नैनोकण-आधारित वितरण प्रणालियों के अंदर दवाओं के इनकैप्सुलेशन ने इन छोटे अणु दवाओं की परिसीमन को दूर करने के लिए एक बड़ी प्रभावकारिता दिखाई है। इस उद्देश्य से, इस थीसिस में MIN6-व्युत्पन्न बाह्यकोशिकीय पुटिकाओं (CEV) का उपयोग करके TEL नैनोफॉर्म्यूलेशन विकसित करना है जो न केवल वितरण माध्यम का कार्य करता है बल्कि चिकित्सीय लाभ प्रदान करता है। इसके अलावा, यह दिखाने के लिए कि CEV चिकित्सीय लाभ प्रदान करता है और TEL लोडेड CEV की प्रभावकारिता की तुलना करता है, हमने सीईवी की लिपिड रचनाओं की नकल करते हुए बायोमिमेटिक लिपिड नैनोकणों (BLN) में TEL को एनकैप्सुलेट करके लिपोटेल को संश्लेषित किया है। हमारे अध्ययन से पता चला है कि नैनोटीईएल के साथ T1DM चूहों का उपचार करने से अग्राशयी सूजन वाले माइक्रोएन्वायरमेंट के मॉड्यूलेशन के माध्यम से अग्राशयी β कोशिकाओं के कार्य को बहाल करके लिपोटेल से बेहतर प्रदर्शन करता है। हालाँकि, नैनोटीईएल के साथ उपचार समय के साथ रक्त शर्करा के स्तर को कम करने में विफल रहा। इसके अलावा, T2DM, जो मधुमेह के कुल मामलों का ~90% है, में बहुआयामी जटिलताएँ हैं। इस प्रकार, T2DM के हाइपरग्लेसेमिक माइक्रोएन्वायरमेंट द्वारा उत्पन्न जटिलता को संबोधित करने के लिए, एक ग्लूकोज-उत्तरदायी जैव-प्रेरित फॉर्म्यूलेशन, जिसे डायबोगेल

कहा जाता है, विकसित किया गया था, जिसमें संशोधित ग्लूकागन जैसे पेप्टाइड-1, TEL और CEV शामिल थे। इन विट्रो और इन विवो प्रभावकारिता विश्लेषण से पता चला है कि डायबोगेल के साथ उपचार T2DM की बहुआयामी जटिलता को संबोधित कर सकता है जिसके परिणामस्वरूप रक्त ग्लूकोज कम हो जाता है, अग्राशयी β सेल कार्यों में सुधार होता है और साथ ही हेपेटोटॉक्सिसिटी भी कम हो जाती है।

Table of contents

Acknowledgments	i
Abstract	iii
Table of contents	viii
List of figures	xi
List of tables	xiii
Abbreviations	xiv
CHAPTER 1: Introduction and literature review	1-33
1.1 Diabetes mellitus (DM) and its types	2
1.2. Pathophysiology of DM	3
1.2.1. Pancreas and their role	3
1.2.2. T1DM	4
1.2.3. T2DM	5
1.2.3.1 Genetic, lifestyle, and environmental factors	
1.2.3.2. Oxidative stress and ROS	
1.2.3.3. DNA damage and repair	
1.2.3.4. Mitochondrial dysfunction and endoplasmic reticulum stress	
1.2.3.5. The impact of nutritional interventions	
1.2.3.6. Insulin resistance (IR)	
1.2.4. GLP-1 mediated insulin secretion	7
1.2.4.1. DPP-4 mediated degradation of GLP-1	
1.2.5. The role of angiotensin II type 1 receptor (AT1R) and its blockers (ARB) in DM	9
1.3. Diabetes associated hepatotoxicity	9
1.4. Treatment modalities available for diabetes treatments	10
1.4.1. Biaguanides	10
	viii

1.4.2. Sulphonylurea	11
1.4.3. Meglitinides	11
1.4.4. Thiazolidinediones	12
1.4.5. Insulin	12
1.4.6. GLP-1 analogues	13
1.4.7. DPP-4 inhibitors	14
1.4.8. α -glucosidase inhibitors	15
1.4.9. SGLT-2 inhibitors	15
1.4.10. Combination therapy	17
1.5. Emerging new technology and future trends	19
1.5.1 Extracellular vesicle	19
1.5.2. Stimuli-responsive delivery systems	20
1.5.3. Nanoformulation for DM	23
1.6. The present thesis	24
1.7. References	26
CHAPTER 2: Telmisartan encapsulated inside extracellular vesicle outperformed biomimetic lipid nanoparticle for the amelioration of diabetes mellitus	34
2.1. Introduction	35
2.2. Experimental Section	36
2.3. Results and discussion	45
2.4. Conclusion	67
2.5. References	67

CHAPTER 3: A glucose-responsive hydrogel laden with modified-GLP-1, Ev, and telmisartan ameliorates type 2 diabetes and reduces hepatotoxicity	77
3.1. Introduction	78
3.2. Experimental Section	80
3.3. Results and discussion	87
3.4. Conclusion	105
3.5. References	106
CHAPTER 4: Summary and future studies	113
4.1. Summary	114
4.2. Future studies	116
List of publications	117
Brief biodata	118

List of figures

Chapter 1

- Figure 1. The figure illustrates various insulin analogues and their positive and negative aspects 13

Chapter 2

- Figure 1. Isolation of CEV, synthesis of BLN, and their physicochemical characterization 45
- Figure 2. Synthesis and physicochemical characterization of TEL-nanoformulations 47
- Figure 3. *In vitro* uptake study 48
- Figure 4. *In vitro* cytocompatibility analysis of TEL, nanoTEL, and lipoTEL determined by MTT assay 49
- Figure 5. Amelioration of in vitro oxidative imbalance 50
- Figure 6. Glucose uptake assay and determination of GLUT4 expression in C2C12 myotubes 52
- Figure 7. Regulation of blood glucose and insulin levels, and restoration of the structure and function of islets of Langerhans 54
- Figure 8. Regulation of inflammation and cellular proliferation in the pancreas of STZ-induced diabetic mice 57
- Figure 9. Modulation of anti-inflammatory / pro-inflammatory cytokines ratio in the serum of STZ-induced diabetic mice 60
- Figure 10. Macrophage population in the pancreas of STZ-induced diabetic mice 63
- Figure 11. Pharmacokinetics and pancreatic concentration of TEL 65
- Figure 12. Hepatotoxicity assay 66

Chapter 3

Figure 1. Synthesis and physicochemical characterization of Diabogel	88
Figure 2. Rheology and release study	89
Figure 3. <i>In vitro</i> cytocompatibility analysis	91
Figure 4. <i>In vitro</i> uptake assay	92
Figure 5. <i>In vitro</i> efficacy of Diabogel	93
Figure 6. <i>In vivo</i> efficacy of Diabogel	95
Figure 7. Diabogel modulated inflammation in HFD+STZ-induced T2DM	98
Figure 8. Diabogel promoted M2 macrophage in HFD+STZ-induced T2DM	100
Figure 9. Diabogel mediated regulation of NF- κ B and PPAR- γ expression, angiogenesis, and cellular proliferation	102
Figure 10. Diabogel lowered HFD+STZ induced hepatotoxicity	104

Chapter 4

Figure 1. Schematic depicting effects of nanoTEL and Diabogel treatments in T1DM and T2DM murine models, respectively	116
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List of tables

Table 1. Different classes of pharmacological drugs for diabetes	15
Table 2. Various stimuli-responsive formulations for the management of DM	21

Abbreviations

DM	Diabetes mellitus
CVD	Cardiovascular disease
WHO	World Health Organization
T2DM	Type 2 diabetes mellitus
IDF	International Diabetes Federation
T1DM	Type 1 diabetes mellitus
PI	Phosphatidylinositol
GLUT	Glucose transporters
GADA	Glutamic acid decarboxylase autoantibodies
ICA	Islet cell autoantibodies
IAA	Insulin autoantibodies
ROS	Reactive oxygen species
AGEs	Advanced glycation end products
PKC	Protein kinase C
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
8-OHdG	8-Hydroxy-2'-deoxyguanosine
IR	Insulin resistance
GLP-1	Glucagon-like peptide-1
DPP-4	Dipeptidyl peptidase-4
cAMP	Cyclic adenosine monophosphate
Epac	cAMP-regulated guanine nucleotide exchange factor
AT1R	Angiotensin II type 1 receptor
ARB	Angiotensin II type 1 receptor blocker

TEL	Telmisartan
HFD	High fat diet
NAFLD	Non-alcoholic fatty acid liver diseases
SGLT-2	Sodium-glucose cotransporter-2
SU	Sulfonylureas
TZD	Thiazolidinediones
NPH	Neutral protamine hagedorn
Ev	Extracellular vesicles
CEV	MIN6-derived extracellular vesicles
GOx	Glucose oxidase
Con A	Concanavalin A
PBA	Phenylboronic acid
NPs	Nanoparticles
BLN	Biomimetic lipid nanoparticles
STZ	Streptozotocin
PPAR- γ	Peroxisome proliferator-activated receptor γ
NF- κ Bp65	Nuclear factor kappa B p 65
HIF-1 α	Hypoxia-inducible factor-1 alpha
FBS	Fetal bovine serum
DMEM	Dulbecco's Modified Eagle's Medium
DiI	1,1-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine Perchlorate
2-NBDG	2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2- Deoxyglucose
DCF-DA	2,7-Dichlorofluorescein diacetate

NIB	National Institute of Biologicals
CryoHRTEM	Cryo high-resolution transmission electron microscopy
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide
IP	Intraperitoneal
IF	Immunofluorescence
ECL	Chemiluminescence
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydrogen
PBS	Phosphate buffer saline
IPGTT	Intraperitoneal glucose tolerance test
ALT	Alanine transaminase
AST	Aspartate transaminase
M2	Anti-inflammatory macrophage
M1	Pro-inflammatory macrophage
mGLP-1	Modified GLP-1
H&E	Hematoxylin and eosin
CM	Conditioned media
NTA	Nanosight tracking analysis
EDC	1-Ethyl-3-diaminopropyl carbodiimide
NHS	N-hydroxysuccinimide
BSA	Bovine serum albumin
SD	Standard deviation
ICG	Indocyanine green
BW	Body weight