UNIVERSITI PUTRA MALAYSIA

THERAPEUTIC EFFICACY OF STEM CELLS (DPSCs, EPCs AND HSCs) WITH TPO-COMBINED TREATMENT FOR DENGUE VIRUS INFECTION IN MICE

SIVAN PADMA PRIYA

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By

SIVAN PADMA PRIYA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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July 2018

Chairman : Suresh Kumar Subbaiah, PhD
Faculty : Medicine and Health Sciences

The debilitating diseases are continuously threatening the humankind and raising the economic burden every year. Dengue is one of the highly extending viral infections spreading beyond the endemic borders. Dengue virus (DENV) infects 200 million people per year and disables 3.6 billion people approximately and the recounted threat to public never decline in spite of advances in medical science. Current treatment modalities are principally limited to symptomatic relief yet, accompanies the bitter side of the side effects, intolerance, and drug interaction especially for the patients with co-morbidities or with confusing co-infections. The majority of their pathogenesis has not been detailed. DENV infections effects are principally reported as dysregulated immune responses targeting all major organs. The DENV infection has been reported to target tissues of the blood vessels, liver and bone marrow manifesting with extensive haemorrhages, reduced platelet count, and finally leading to the fatal end in selected individuals. The regenerative medicine has evolved with more promising results with stem cells therapy (SCT). The SCT can be defined as delivering or transplanting primitive cells of the damaged organ or tissue to replace and to augment roles of the injured cells. There were few reports regarding SCT for infections like human immunodeficiency virus and for endotoxin-induced sepsis. The paramount of research on the ability of stem cells with immunomodulatory and anti-apoptotic activities have strengthened the idea of novel therapeutic approaches. The principal aim of this study is to investigate the therapeutic ability of dental pulp stem cells (DPSCs) from human cell line along with endothelial progenitor cells (EPCs) of mouse and haematopoietic stem cells (HSCs) of mouse to repair and regenerate the damages induced by DENV2 infection. There were 72 male BALB/c mice brought at the age of eight weeks and were grouped into three as control, infected and not treated with stem cells (Non-SCT), and infected and stem cells treated (SCT). The control was injected with eagle minimum essential media (EMEM) and phosphate buffer

saline (PBS). The Non-SCT group mice were infected with DENV2; SCT group were infected with DENV2 and treated with DPSCs, EPCs, and HSCs with a growth factor thrombopoitin. Both the Non-SCT and SCT group were injected intraperitoneally (IP) with DENV2 for consecutive two days. The mice were sacrificed at 5, 10, 15, and 21st day of post-infection by terminal anaesthesia and blood was collected by cardiac puncture to perform haematological and biochemical analysis and the tissues were preserved for histopathological analysis. The Non-SCT group exhibited the marked reduction in platelets, alterations in the liver enzymes and severe pathological damages in the liver and blood vessels. The SCT group exhibited a lesser degree of involvement throughout the experiment and evidenced better recovery by the 21st day. The therapy has aided the recovery and regeneration of the important altered manifestation of dengue such as recovered platelet count, reduced cellular damages, reduced apoptosis, reduced viral load by the end of the 21st day of the experiment. The changes were more significant after the 15th day when the recovering body system suffers from the loss of cells due to extensive viral induced damages which were controlled in SCT group. The finding can conclude the efficacy of the stem cell combination treatment in aiding the alleviation of the dengue induced damages.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEBERKESANAN TERAPEUTIK DARI KOMBINASI SEL STEM (DPSCs, EPCs & HSCs) DENGAN TPO SEBAGAI RAWATAN BAGI JANGKITAN VIRUS DENGGI PADA TIKUS

Oleh

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Penyakit yang melemahkan terus mengancam manusia dan meningkatkan beban ekonomi setiap tahun. Denggi merupakan salah satu daripada jangkitan virus yang menyebar luas secara melampau di luar sempadan endemik. Virus Denggi (DENV) menjangkiti 200 juta orang setahun dan melumpuhkan kira-kira 3.6 bilion orang dan ancaman terhadap umum tidak pernah merosot walaupun berlaku kemajuan dalam sains perubatan. Modul rawatan semasa, pada dasarnya hanya terhad kepada pelepasan gejala, yang diiringi oleh kesan sampingan yang perit, intoleransi, dan interaksi ubat terutamanya untuk pesakit yang mengidap penyakit atau dengan jangkitan bersama yang mengelirukan. Majoriti patogenesis mereka tidak terperinci. Kesan jangkitan DENV pada dasarnya dilaporkan sebagai tindak balas imun yang diselaraskan menyasar semua organ utama. Jangkitan DENV telah dilaporkan menyasar tisu saluran darah, hati dan sumsum tulang yang dimanipulasikan dengan pendarahan yang meluas, pengurangan jumlah platelet, dan akhirnya membawa maut pada individu terpilih. Perubatan regeneratif telah berkembang dengan hasil yang lebih menjanjikan dengan terapi sel stem (SCT). SCT boleh ditakrifkan sebagai menyampaikan atau memindahkan sel primitif kepada organ atau tisu yang rosak untuk menggantikan dan menambah peranan sel-sel yang cedera. Terdapat beberapa laporan mengenai SCT untuk jangkitan seperti virus kurang imun manusia dan sepsis yang disebabkan oleh endotoxin. Kebanyakan penyelidikan tentang keupayaan sel stem dengan aktiviti imunomodulator dan anti-apoptosis telah memperkuat idea kami tentang pendekatan terapi baru. Matlamat utama kajian kami adalah untuk mengkaji keupayaan terapeutik sel stem pulpa gigi (DPSCs) daripada manusia, bersama selleluhur endothelial (EPCs) daripada tetikus dan sel stem haematopoietik (HSCs) daripada tetikus untuk membaiki dan memperbaharui kerosakan yang disebabkan oleh jangkitan DENV2.Terdapat 72 tikus BALB / c jantan yang dibeli pada usia lapan minggu dan dibahagikan menjadi

tiga kumpulan sebagai kawalan (Control), dijangkiti dan tidak dirawat dengan sel stem (Non-SCT), dan yang dijangkiti dan dirawat dengan sel-sel stem (SCT). Kawalan disuntik dengan media "eagle minimum essential media" (EMEM) dan "phosphate buffer saline" (PBS). Kumpulan Non-SCT dijangkiti DENV2; Kumpulan SCT dijangkiti DENV2 dan dirawat dengan DPSCs, EPCs, dan HSCs dengan factor pertumbuhan. Kedua-dua kumpulan Non-SCT dan SCT disuntik secara intraperitoneal (IP) dengan DENV2 selama dua hari berturut-turut. Tikus telah dikorbankan pada 5, 10, 15, dan 21 hari selepas jangkitan dengan menggunakan anestesia terminal dan darah dikumpulkan melalui tusukan jantung untuk analisi hematologi dan biokimia dan tisu-tisu dipelihara untuk analisis histopatologi. Kumpulan Non-SCT menunjukkan pengurangan platelet yang jelas, perubahan dalam enzim hati dan kerosakan patologi yang teruk di hati dan saluran darah. Kumpulan SCT menunjukkan tahap penglibatan yang lebih rendah sepanjang eksperimen dan membuktikan pemulihan yang lebih baik pada hari ke-21. Terapi ini telah membantu pemulihan dan pertumbuhan semula manifestasi penting bagi demam denggi seperti kembalinya platelet, mengurangkan kerosakan selular, mengurangkan apoptosis, mengurangkan zahrah virus pada hari ke-21experimen. Perubahan itu lebih signifikan selepas hari ke-15 apabila system pemulihan badan mengalami kehilangan sel-sel disebabkan kerosakan yang banyak oleh virus yang dikawal dalam kumpulan SCT. Penemuan ini dapat menyimpulkan keberkesanan kombinasi sel stem dalam membantu pengurangan kerosakan akibat denggi.

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I certify that a Thesis Examination Committee has met on 11 July 2018 to conduct the final examination of Sivan Padma Priya on her thesis entitled "Therapeutic Efficacy of Stem Cells (DPSCs, EPCs and HSCs) with TPO-Combined Treatment for Dengue Virus Infection in Mice" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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α	Alpha
β	Beta
γ	Gamma
λ	Lambda
ADE	Antibody-dependent Enhancement
ALI	Acute Lung Injury
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AP-1	Activator Protein-1
APC	Allophycocyanin
APCs	Antigen Presenting Cells
APTT	Activated partial prothrombin time
AST	Aspartate transaminase
AT	Aminotransferases
ATTC	American Type Culture Collection
b 1 integrin	Beta-1 Integrin
B-cells	B-Lymphocytes
Bc	Bile Canaliculi
BD	Bile Duct
BM	Bone-marrow
BMMCs	Bone marrow-derived Mononuclear Cells
BNC	Binucleated Cells
BV	Blood Vessels
C	Capsid

CD	Cluster of differentiation
CEC	Circulating endothelial cells
CF	Collage Fibres
CFU	Colony forming units
CHIKV	Chikungunya Virus
c-kit	Cellular kit
CLRs	C-type Lectin Receptors
CMC	Carboxymethyl cellulose
CNCC	Cranial Neural Crest Cells
CO^2	Carbon dioxide
CPE	Cytopathic effect
Cv	Central Vein
CXCL	CXC chemokine ligand
d.p.i	Day of post infection
ds	Double Strand
DAB	3,3'-Diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole (Stain)
DCs	Dendritic cells
DC-SIGN	Dendritic cell-specific Intercellular adhesion molecule-3-grabbing non-integrin
DENV	Dengue Virus
DENV1	Dengue Virus Serotype 1
DENV2	Dengue Virus Serotype 2
DENV3	Dengue Virus Serotype 3
DENV4	Dengue Virus Serotype 4
DF	Dengue Fever

DHF	Dengue haemorrhagic fever
DIC	Disseminated Intravascular Coagulation
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPSC	Dental Pulp Stem Cells
DSC	Dental Stem Cells
DSS	Dengue shock syndrome
ECD	Effective Cell Dose
ECs	Endothelial cells
ECM	Extracellular matrix
EDTA	Ethylene diamine tetra acetic acid
EGF	Epidermal growth factors
EL	Elastic Lamina
EMEM	Eagle minimum essential media
EMT	Epithelial to Mesenchymal Transition
EPCs	Endothelial progenitor cells EPO Erythropoietin
ER	Endoplasmic Reticulum
ESCs	Embryonic stem cells
F	Fibres
Fas	transmembrane protein of TNF family
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FcγII	Fc gamma 2
FcγRII	Fc gamma receptor 2
FDA	Food and Drug Administration

FFA	Foci forming assay
FFU	Foci forming Units
FGF	Fibroblast growth factors
FITC	Fluorescein isothiocyanate
FLT	fms-like tyrosine kinase
G	Gauze
HB	Haemoglobin
HBV	Hepatitis B Virus
Hc	Hepatocytes
HCT	Haematocrit
H&E	Hematoxylin and Eosin
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HP	Histopathology
HSCs	Hematopoietic stem cells
HSPC	Haematopoietic stem progenitor cells
iPSCs	Induced pluripotent stem cells
IA	Intra-Arterial
IACUC	Institutional animal care and use committees
Ic	Ito cells
ICAM	Intercellular adhesion molecule
ICTV	International Committee on Taxonomy of Viruses
IEL	internal Elastic Lamina
IFN	Interferon
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IL	Interleukin
IMR	Institute for medical research
IP	Intraperitoneal
IUL	Intrauterine life
IV	Intravenous
kb	Kilobase
KCs	Kupffer cells
kDa	Kilodalton
KIR	Killer cell immunoglobulin-like receptor
LFT	Liver Function Test
Lin	Lineage
LT-HSCs	Long term- Haematopoietic Stem cells
mAb	Monoclonal antibodies
Mac-1	Macrophage-1 antigen
MAPCs	Multipotent Adult Progenitor Cells
MCP	Monocyte Chemoattractant protein
MEP	Megakaryocyte–erythroid progenitor
MET	Mesenchymal to Epithelial Transition
MHC	Major Histocompatibility Complex
MIF	Macrophage Migration inhibitory factor
MNC	Mononucleated Cells
MMP	Matrix metallopeptidase
MPP	Multipotent progenitors
MSCs	Mesenchymal stem cells
MTc	Masson Trichrome (Stain)

NAFLD	Non-alcoholic fatty liver disease
NF	No foci
NF-E2	Nuclear factor, erythroid-2
NF- κB	Nuclear factor kappa B
NK cells	Natural Killer cells
NLRs	NOD-like Receptors
Non-SCT	Not treated with Stem Cell treatment
NP-40	Nonyl phenoxypolyethoxylethanol 40
NS protein	Non-structural protein
NSC	Neuronal Stem Cells
PAIgM/PAIgG	Platelet associated immunoglobulins
PAS	Periodic Acid Schiff reagent (Stain)
PBS	Phosphate buffer saline
PBSCs	Peripheral Blood Stem Cells
PCV	Packed Cell Volume
PDGF	Platelet-derived growth factor
PE	Phycoerythrin
PFU	Plaque Forming Units
PLT	Platelets
PMID	PubMed-Indexed
PMN	Polymorphonuclear
Pp	Polyploidy
prM	Pre-membrane
PRR	Pattern Recognition Receptors
PT	Prothrombin time
RBC	Red blood cells

RBP4	Retinal-binding Protein 4
RLRs	RIG-I-like Receptors
ss	single strand
S	Sinusoids
SCs	Stem Cells
Sca	Stem cells antigen
SCF	Stem cell factor
SCT	Stem Cell Treatment
SDF	Stromal cell-derived factor
SPF	Specific Pathogen free
SSC	Somatic Stem Cells
ST-HSCs	Short term- Haematopoietic Stem cells
T-cells	T-Lymphocytes
TAG	Triacylglycerol
TCID50	Tissue culture infective dose assay
Te	Tunica externa
TEM	Transmission Electron Microscope
TGF	Transforming growth factor
Ti	Tunica intima
TLR	Toll-like Receptors
Tm	Tunica media
TNF	Tumour necrosis factor
TNFR	Tumour necrosis factor receptor
TNTC	Too numerous to count
TPO	Thrombopoietin
TRAIL	TNF-related apoptosis-inducing ligand

TSG	TNFα-stimulated gene-6
UKM	Universiti Kebangsaan Malaysia
UPM	Universiti Putra Malaysia
UPR	Unfolded Protein Response
VCAM-1	Vascular Cell Adhesion Molecule 1
VECs	Vascular Endothelial Cells
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
VSMCs	Vascular Smooth Muscle Cells
vWF	Von Willebrand factor
WBCs	White blood cells
WHO	World Health Organization
WPBs	Weibel–Palade bodies

CHAPTER 1

INTRODUCTION

1.1 Background

Upkeep of good health has always been a major wish for any individual in the world. Health has been considered as a major factor by the United Nations Agenda 2030 signed by 193 Governments across the world aiming at transforming the World for Sustainable Development. Among the 16 goals, Goal 3 aims to "Ensure healthy lives and promote well-being for all of all ages." The contribution of medical personnel is inevitable for this lofty ideal. However newer and dreadful challenges are confronted by human population in different parts of the world with regard to endemic as well as new disease patterns through new strains of viruses making constant observation and development of new treatment strategies through medical research an inseparable and vital component of medical profession throughout the world.

Science has revolutionized to intrude the atomic or nanomolecular level of many subjects, including human tissues (Pearson et al. 2002). Regenerative medicine and tissue engineering field of medicine have developed to the level of printing three-dimensional tissue with functional cells to replace the damaged tissues (Pearson et al. 2002). But, the challenges are consistent with the countless degenerative disorders and by the debilitating infectious diseases. Evolving microbial diseases constantly threaten the humankind. Viruses, possessing few base pairs of genomic material have the ability to sneak inside the cellular and nuclear level of the human cells owning few million base pairs of the genome and can sway them at the molecular level

Viral outbreaks in endemic areas trigger panic among the world populace because of the upsetting upsurge in infection spreading speed due to the reduced travelling speed and many other multifactorial facts exploiting the host defencelessness. The dengue virus infection is the most prevalent and rapidly spreading mosquito-borne viral infection (Guzman and Harris 2015) and has been reported as the second commonest cause for hospitalization of tropical travellers in Europe next to malaria (Bhatt et al. 2013). The DENV (dengue virus) infects 200 million per year and disable 3.6 billion people approximately and the recounted thread to public never decline in spite of advances in medical therapeutic science (Thisyakorn and Thisyakorn 2015). There are four serotypes of dengue (DENV1, DENV2, DENV3 and DENV4) verified impose enormous research works to achieve the control, but emergences of new fifth type threads the future (Mustafa et al. 2015). DENV2 is one of the highly virulent and currently predominant serotypes compared to all the other four dengue types (Cucunawangsih and Lugito 2017, van Panhuis et al. 2010).

DENV manifestations vary from subclinical presentation to devastating disease with disastrous outcome revealing mortality rate of 5% to 30% (Liew et al. 2016). DENV largely targets and causes maximum damages to blood vessels (BV) chiefly on the vascular endothelial cells (VECs) and its supporting structures (Malavige and Ogg 2017, Yacoub et al. 2013). Liver owning dual circulation comprising widespread sinusoidal spaces lined by VECs, is also extensively damaged by DENV (Paes et al. 2005). The crucial issues of severe DENV infection are due to the dysregulated immune response leading to extensive bleeding resulting with hypovolemia, which was not responding to the fluid and platelet replacement rather worsening the situation (Malavige and Ogg 2017). Treatment outcomes with corticosteroids to modulate the immune system were inconclusive and not widely accepted (Zhang and Kramer 2014). Intravenous immunoglobulin was suggested for a clinical trial with limited evidence (Rajapakse 2009). A tetravalent vaccine against all the serotypes of DENV is still under the elaboration and need to meet successful worldwide clinical use (Flasche et al. 2016, Remy 2014, Chokephaibulkit and Perng 2013).

There is an alarming 30-fold increase in a number of dengue cases reported from 1960 (Fitzpatrick et al. 2017). The associated economic burden raises billions of dollars every year (Fitzpatrick et al. 2017). There is no accepted treatment with synthetic or natural medicines existing which is widely accepted and cost-effective for prophylaxis and treatments, and similarly, there is no existing cost-effective, standard and approachable vaccination against all the four types of DENV infections (Flasche et al. 2016). There is an insistent need for novel treatment to be developed for the safe future.

Alternatively, a therapy based on the need of the host has been designed. DENV infection is immunologically controlled by the host within the stipulated time and life-long immunity is developed, but the burden is due to the dysregulation of host immune system and the protein reservoir liver is also overburdened which influences the repair and regeneration. While the immunity progressing with its predictable effort, if it is possible to deliver the essential cells, the untoward effects of DENV infections can be nullified. The novel prospect is currently rising from the regenerative medicine with stem cell therapy (SCT) unambiguously, which has proven its efficacious in many diseases for example in liver disease by delivering the replacement cells without genetic modification (Ohkoshi et al. 2017).

The notion of using SCs to aid better recovery of the chronic and complicated infections have been initiated (Zhang et al. 2014) but not yet progressed to reach the clinical application. Infections either acute or chronic, which are fulminating, hassle the human system during repair and recovery (Reis 2017). Some do leave permanent tissue damage in vital organs, even after the infection was abolished utterly, which compromises the general physiological functions for rest of the life (van den Pol 2009). Studies targeting the ability of mesenchymal stem cells (MSCs) to regress the inflammation and infection have proved the success of antimicrobial action of MSCs against the bacterial infections such as *Pseudomonas aeruginosa*, *Staphylococcus*

aureus, and *Streptococcus pneumoniae*, by producing antimicrobial peptide LL-37 (Sutton et al. 2016).

Cellular therapy with BM (bone-marrow)-derived mononuclear cells for experimentally induced poly-microbial sepsis in mice concluded that the early delivery has beneficial effect probably by their paracrine effect (Ornellas et al. 2011). BM-derived SCs have improved the lung function in experimentally induced malaria in mice (Souza et al. 2015). The success and future suggestions regarding cellular therapy to a parasitic infection (Wang et al. 2015) and a bacterial infection (tuberculosis) (Parida et al. 2015) were reported. But SCT for DENV infection has never been affirmed. The immunomodulatory action, plasticity and multi-lineage properties of the SCs made the regenerative therapy to reach the paramount success within few years (Caplan and Correa 2011). The SCs of autologous origin attracted the concept of "personalized treatment" (Lin et al. 2016). MSCs initiated with infections treatment, are devoid of drug-related side effect as an autologous origin with high potential to repair and regenerate (Vassilopoulos et al. 2003).

MSCs were first demonstrated in BM as a type of adult SCs which do not belong to haematopoiesis (Friedenstein et al. 1970) and later demonstrated in many organs including dental pulp stem cells (DPSCs) (Gronthos et al. 2000). DPSCs are one of the promising sources, easily accessible and available at all the ages (Egusa et al. 2012). The immunomodulatory effect of DPSCs through various mechanisms has been researched (Yang et al. 2017, Li, Jiang, et al. 2014, De Miguel et al. 2012), which can be alternatively used to control the infections. Dental stem cells (DSCs) possess substantial benefits over the other sources of MSCs such as, the non-invasive collection of cells from a medically wasted extracted tooth (Egusa et al. 2012); hundred times high proliferation rate than BM-MSCs (Gronthos 2011), and an extended life without undergoing senescence during laboratory expansions and storage (Bakopoulou and About 2016). DPSCs' outstanding combined competency of strong angiogenic and neurogenic ability strengthens their decision of usage for DENV infections (Feng et al. 2013). The BVs are closely intermingled with the sympathetic nervous system which controls their development and their effective function. While angiogenesis is the primary step in any tissue repair, neurogenic stimuli by their transmitters are important for angiogenesis (Pan et al. 2016). DPSCs being derived from the cranial neural crest cells (CNCC), which is also responsible for the development of tunica media and tunica externa of the major blood vessel (abdominal aorta) (Achilleos and Trainor 2012), the DPSCs have close communication with the sympathetic nerves during homeostasis (Shi and Gronthos 2003) (Byers et al. 2003). DSCs have the ability to regenerate into many different types of cells including liver cells (Ranganathan and Lakshminarayanan 2012).

The SCs related to the BV (blood vessels) are endothelial progenitor cells (EPCs) and the MSCs. The depleted platelets due to the bleeding episodes are needed to be restored by the haematopoietic SCs (HSCs) (Ng and Alexander 2017, Vassilopoulos et al. 2003). The differentiation of megakaryocytes, the creator of platelets is

stimulated by the thrombopoietin (TPO) (Haegele et al. 2015). The liver hepatocytes are derivatives of MSCs (Zarrinpar and Busuttil 2013). SCT for DENV needs a perfect blend of SCs for effective repair and regeneration of the injured structures, which can be achieved by a combination of the SCs including MSCs, EPCs, and HSCs with TPO.

1.2 Problem statement

Despite the urgencies regarding the control and treatment of DENV infections, the pathogenesis remains insubstantial (Paes et al. 2005). The DENV infected host does develop lifetime immunity but restricted to the particular serotype. Consecutive DENV infection with another serotype might end with the worst situation due to original antigenic sin, which was due to the cross-reaction of the antibodies developed for the first infection binding ineffectively with the antigens of new infections and leaving them uninhibited (Zompi and Harris 2013). The approval of tetravalent dengue vaccine, Dengvaxia by 2015 in Mexico has provided protection but appears to be limited in time and serotype, especially against the DENV2 which is less protected (Sim and Hibberd 2016, Capeding et al. 2014). There are no reports about cross-reaction of commercially available dengue vaccines. Emerging clinical reports alarms the troubles of post-infection complications and co-infections, and co-morbidities accumulating the fatalities (Priya et al. 2017, Remy 2014).

There are many fatal infections lead to post-infection complications including DENV and lack of enough renewal cells during repair due to the extensive involvement (Espinoza et al. 2017, Yacoub et al. 2013, Teng and Chatham 2015, Horrobin 1990). The use of immunosuppressive or immunomodulatory medicine have been suggested but the currently available drugs do possess strong contraindication during viral infections (Chawla, Yadav, and Chawla 2014). The immunomodulatory effect of SC is expected to aid and prevent the immune-related damages (Malavige and Ogg 2017, Gao et al. 2016).

The healing of any diseases is encouraged with supplementary treatment modalities with proteins, vitamins, and minerals to aid the existing SCs to form the new healthier cells, but the notion of providing SCs as an adjuvant is an appreciable alternative as regenerative repair. The medicinal cure with antibiotics; antiviral; anti-parasitic; anti-metabolites and immunomodulatory drugs were challenged with side effects, resistance, and many other untoward results (Stefano et al. 2017, Vcev 2009). The drugs aid the host by reducing the pathogen load and support the repair by bringing better milieu for the existing SCs but do not supply renewing cells. The advances in regenerative therapy promise the alternative therapy for infectious diseases by providing renewing cells (Thanunchai, Hongeng, and Thitithanyanont 2015). The prodigious success of BM-derived SCs to aid the BM-cancers paved a way to other cancer therapies and for chronic illnesses such as degenerative disorders; autoimmune diseases; Graft Versus Host Diseases and ageing problems (Rohban and Pieber 2017). SCs ability to modify the role of many defence cells including T cells, B cells, dendritic cells (DCs) and natural killer (NK) cells strengthens the notion for infection

therapy (Ma and Chan 2016, Ho, Mei, and Stewart 2015). The success of any therapeutic innovation in the medical profession is achieved only when it reaches the clinical trials. There is severely lagging in the therapeutic application pathway of SCs from "lab to life" which need to be completed.

The fatal manifestations including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (da Cunha and de Oliveira 2012). Alternative therapy suggestion with SCs in the field of infection therapy include, the human immunodeficiency viral infection (Rabson 2013, Allers et al. 2011, Hutter et al. 2009); malarial parasitic infection (Wang, Qian, and Cao 2015); bacterial infection (Parida et al. 2015, Skrahin et al. 2014, MacMicking 2014, Skrahin et al. 2016), and sepsis (Mei et al. 2010, Ho, Mei, and Stewart 2015). SCT for repairing damaged vital organs like lung, kidney and in the liver, have reported by many research (Akram et al. 2016, Souza et al. 2015, Hashemi Goradel et al. 2015, Kotton and Morrisey 2014, Zhu, Lerman, and Lerman 2013, Meier et al. 2013, Hoffman et al. 2011) but not for dengue infections.

One of the potent sources of MSCs is DPSCs (Rai, Kaur, and Kaur 2013, Potdar and Jethmalani 2015) which can induce angiogenesis (Bronckaers et al. 2013) and differentiate into hepatocytes (Ohkoshi et al. 2017) have been reported, but not in the DENV infections (Potdar and Jethmalani 2015). There was a constant search for improving the efficiency of the stem cells during therapy. The avenues suggested including increasing the trafficking by chemotactic agents and improving growth factor production (Ichim et al. 2010). But stem cell combination is a novel notion. The MSCs provide the better microenvironment for the required SCs and promote the cell expansion with synergetic ability as reported with animal studies (Ichim et al. 2010, Hosseini, Farahmandnia, et al. 2015, Urban et al. 2008). The SCs combination was preferred to improve the efficacy, which needs to be evaluated. The study is about experimenting the potential of dental tissue-derived SCs in aiding the repair and regeneration of body tissues in one of the communicable diseases, DENV2 infection, along with EPCs, and HSCs by validating their ability to repair and regenerate the damaged tissues due to infection. The novel approach in SCT in dengue infection aims to revolutionaries the therapeutic approach for many communicable diseases of never-ending social burden.

The success of SCT needs to be evaluated through experimental studies by analysing their mechanism of action in the tissues at the cellular and molecular level; possible reasons for the better outcome and their future applications, and the possibilities of any untoward effects by comparing the structural changes and other investigations and their possible rectifications. The research question had been set to open a new era in longstanding quarries in the treatment of viral infections with SCs and to explore their validity with empirical data produced with the biochemical, histopathological, ultrastructural and immunological techniques, by exploring all positive and negative results.

1.3 Objectives

1.3.1 General objective

To validate the efficacy of therapy using the combination of the SCs (DPSCs, EPCs and HSCs) with TPO in repairing and regenerating the structural damage in the liver and aorta following the DENV2 infection.

1.3.2 Specific objectives

i. To evaluate the efficacy of SCs (DPSCs, EPCs and HSCs) with TPO combined treatment in restoring the haematological parameters altered by the DENV2 infection
ii. To estimate the ability of SCs (DPSCs, EPCs and HSCs) with TPO combined treatment in re-establishing the liver functions, disordered by the DENV2 infection by assessing the biochemical assays.
iii. To evaluate the ability of SCs (DPSCs, EPCs and HSCs) with TPO combined treatment in rescuing the histopathological changes induced by DENV2 infection by evaluating the tissue-sections of the liver and aorta stained with different stains.
iv. To analyse the ability of SCs (DPSCs, EPCs and HSCs) with TPO combined treatment in reducing the DENV2 load by evaluating the viral antigen in the tissue-sections of the liver and aorta with immunofluorescence.
v. To analyse the efficacy of SCs (DPSCs, EPCs and HSCs) with TPO combined treatment by analysing the ultrastructural details of the liver and the aorta.

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