UNIVERSITI PUTRA MALAYSIA

SYSTEMATIC REVIEW ON THE THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL EXOSOMES FOR TREATMENT OF ALZHEIMER DISEASE

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ABSTRACT

SYSTEMATIC REVIEW ON THE THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL EXOSOMES FOR TREATMENT OF ALZHEIMERS DISEASES

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Introduction: Alzheimer disease (AD) remains the most predominant neurodegenerative disease as it has no definitive cure. The management of the disease relies on the administration of drugs to subside the symptoms. In recent studies, mesenchymal stem cell (MSC)-exosomes have been marked to possess therapeutic potential for treating AD. These exosomes are naturally occurring nanospheres that protect, transport, and deliver bioactive molecules from stem cells that mediate intercellular communication hence regulating target cell function, including the brain cells. Therefore, knowledge of the properties and mode of action of these MSC-derived exosomes in potentially mitigating AD pathogenicity is essential. **Objective:** This study aims to gather findings that focus on the therapeutic potential of MSC-derived exosomes by systematically reviewing and analyzing published works on these exosomes targeting AD. It is hypothesized that MSC-exosomes exhibit high therapeutic potential for AD treatment by exerting various modes of action. **Methodology:** Relevant published works from January 2016 until December 2020 were searched using three databases: PubMed, Scopus, and Medline, using "Alzheimer disease", "secretome", "exosomes", "extracellular vesicles", and "cell-free therapy" as the keywords. Only research articles on MSC exosomes related to AD were selected as the inclusion criteria. Review papers and exosomes of other types of stem cells were excluded. The articles which met the exclusion/inclusion criteria were selected for analysis. Risk of bias was assessed using Office of Health Assessment and Translation tool (OHAT). **Results and Discussion:** A total of 11 eligible *in vivo* and *in vitro* studies were included in this review. Bone marrow-derived stem cells (BMSCs) were the most used source for exosomes isolation. Based on OHAT risk of bias tool, the studies presented various levels of biases. The studies revealed different action modes of MSC-exosomes to alleviate AD pathology. These modes of action include mitigating neuroinflammatory response by reducing oxidative stress and immunomodulation of pro-inflammatory/anti-inflammatory factors, degrading Aβ plaques via the act of degrading enzymes and regulating apoptosis through the delivery of micro-RNAs (miRNAs). **Conclusion:** Findings from this review provided systematic convincing evidence highlighting the therapeutic properties of MSC-derived exosomes as a prospective source for cell-free (acellular) therapy in treating Alzheimer disease.

Keywords: Alzheimer's disease, secretome, exosomes, cell-free therapy, extracellular vesicles

ABSTRAK

ULASAN SISTEMATIK BERKAITAN POTENSI TERAPEUTIK SEL INDUK MESENKIMA BAGI RAWATAN PENYAKIT ALZHEIMER

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Pengenalan: Penyakit Alzheimer (AD) adalah penyakit neurodegeneratif yang paling banyak dihidapi kerana ianya tidak mempunyai rawatan yang tetap. Pengurusan penyakit ini bergantung kepada pemberian ubat yang bertujuan untuk merawat simptom sahaja. Dalam kajian terkini, eksosom dari sel induk mesenkima (MSC) telah dikenalpasti mempunyai potensi terapeutik bagi merawat AD. Eksosom ini merupakan nanosfera yang terjadi secara semulajadi, berfungsi untuk melindungi, mengangkut dan menghantar molekul bioaktif dari sel induk yang menjadi pengantara bagi komunikasi antara sel, lantas, mengawal selia fungsi sel sasaran, termasuklah sel otak. Oleh itu, pengetahuan berkenaan sifat-sifat dan cara tindakan eksosom dari MSC dalam meringankan kepatogenan AD adalah penting. **Objektif:** Kajian ini bertujuan untuk mengumpul hasil dapatan yang memfokuskan kepada potensi terapeutik eksosom dari MSC dengan memberi ulasan secara sistematik dan menganalisis hasil-hasil karya yang telah diterbitkan berkaitan eksosom dalam menyasarkan AD. Secara hipotesisnya, eksosom dari MSC menunjukkan potensi terapeutik yang tinggi dalam merawat AD dengan menggunakan cara tindakan yang pelbagai. **Metodologi:** Hasil karya terbitan dari Januari 2016 sehingga Disember 2020 telahpun dikesan menggunakan tiga pangkalan data: Pubmed, Scopus dan Medline, melalui penggunaan "penyakit Alzheimer", "sekretom", "eksosom", "vesikel ekstrasellular", dan "terapi tanpa sel" sebagai kata kunci. Artikel penyelidikan berkenaan eksosom dari MSC yang berkaitan dengan AD sahaja telah dipilih sebagai kriteria serta. Manakala, kertas ulasan dan eksosom dari jenis sel induk yang lain disingkirkan. Artikel yang menepati kriteria serta dan singkir telah dipilih untuk analisis. Risiko bias telah ditaksir menggunakan *Office of Health Assessment and Translation tool (OHAT)*. **Keputusan dan Perbincangan:** Sebanyak 11 kajian *in vivo* dan *in vitro* yang layak telah dimasukkan dalam ulasan ini. Sel induk dari sumsum tulang merupakan sumber yang paling banyak digunakan untuk pengasingan eksosom. Mengikut alat risiko bias OHAT, kajian-kajian tersebut mengemukakan tahap bias yang pelbagai. Kajian-kajian tersebut juga menunjukkan pelbagai cara tindakan telah digunakan oleh eksosom dari MSC untuk mengurangkan patologi AD. Cara tindakan ini termasuklah meringankan respons keradangan saraf dengan mengurangkan stres oksidatif dan modulasi imun terhadap factor pro/anti-keradangan, degradasi plak Aβ melalui tindakan enzim perosak dan mengawal selia apoptosis melalui penghantaran RNA mikro (miRNAs). **Kesimpulan :** Hasil dapatan dari ulasan ini menyediakan bukti sistematik dan kukuh yang mengfokuskan sifat-sifat terapeutik eksosom dari MSC sebagai sumber prospektif bagi terapi tanpa sel (asellular) dalam merawat penyakit Alzheimer.

Kata kunci: Penyakit Alzheimer, sekretom, eksosom, terapi tanpa sel, vesikel ektrasellular

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LIST OF ABBREVIATIONS

5XFAD	5X Familial Alzheimer Disease
Aβ	Amyloid Beta
ACh	Acetylcholine
AD	Alzheimer Disease
A1AD	Alpha-1 antitrypsin deficiency
ADAM10	A Disintegrin and metalloproteinase domain-containing protein 10
Alix	Programmed cell death 6-interacting protein
APP/PS1	Amyloid precursor protein/presenilin 1
Arg-1	Arginase 1
BACE1	Beta secretase
BCA	Bicinchoninic acid assay
BMP	Bone morphogenetic proteins
BV2	Cellosaurus cell line BV-2
C57BL/6	C57 black 6
C83, C99	C Terminal fragments 83 and 99
Ca_2	Calcium
CD9	Cluster of Differentiation 9
CD14	Cluster of Differentiation 14
CD63	Cluster of Differentiation 63
CD81	Cluster of Differentiation 81
CD90	Cluster of Differentiation 90
DCX	Doublecortin

DDP	Dystonia Peptide
DMP1	Dentin Matrix Acidic Phosphoprotein 1
EE	Early Endosome
eEF1A1	Eukaryotic elongation factor 1A
eEF2	Eukaryotic elongation factor 2
EGF	Epidermal growth factor
ESCs	Embryonic Stem Cells
FDA	Food and Drug Administration
FIZZ1	Resistin-like molecule alpha 1
GFAP	Glial fibrillary acidic protein
GTPase	Guanosine Triphosphate hydrolase enzymes
H_2O_2	Hydrogen Peroxidase
HSP90, HSP60	Heat Shock Protein 90 and 60
Iba1	Ionized Calcium Binding Adaptor Molecule 1
ICV	Intracerebroventricular Injection
IDE	Insulin-degrading enzyme
IL-10	Interleukin 10
iNOS	Inducible Nitric Oxide Synthase
iPSCs	Induced Pluripotent Stem Cells
IV	Intravenous Injection
LTD	Long-Term Depression
LTP	Long-Term Potentiation
M1	Classical Activation
M2	Alternative Activation
MAP	Microtubule Associated Proteins

MSCs	Mesenchymal Stem cells
mTOR	Mechanistic Target of Rapamycin
MVBs	Multivescilar body
MWM	Morris water maze
NEP	Neprilysin
NFT	Neurofibrillary tangles
NMDA	N-methyl D-aspartate
NSCs	Neural Stem Cells
NTA	Nanoparticle Tracking Analysis
OHAT	Office of Health Assessment and Translation
pcDNA3-EGFP	P-complementary Deoxyribonucleic Acid 3- Enhanced Green Fluorescent Protein
PEG	Polyethylene Glycol
PICO	Population, Intervention, Control, and Outcomes
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSA-NCAM	Polysialylated-Neural Cell Adhesion Molecule
PTEN/PI3K	Phosphatase and Tensin homolog/ Phosphoinositide 3-Kinase
QT-PCR	Quantitative Transcription- Polymerase Chain Reaction
SH-SY5Y	Human Neuroblastoma Cell Line
TEM	Transmission Electron Microscopy
TGF-β	Transforming Growth Factor Beta
TGFBR3	Transforming Growth Factor Beta Receptor 3
TSG101	Tumour Susceptibility Gene 101
VEGF	Vascular Endothelial Growth Factor

Ym-1	Chitinase-Like 3

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CHAPTER 1

INTRODUCTION

1.1 Background

Alzheimer disease (AD) remains as one of the most significant neurodegenerative disorders; based on World health organization (2020), nearly 70% of dementia cases are from Alzheimer's disease. According to Alzheimer's disease Foundation Malaysia (2016), it has been estimated that the total number of Malaysian individuals presented with AD was approximately 50,000 in 2016. AD is a progressive, irreversible disease-causing cognitive impairment and memory loss. Multiple hypotheses have been proposed for AD's pathophysiology, including the amyloid cascade hypothesis and the tau protein hypothesis.

For AD, current treatment options such as cholinesterase inhibitors and NMDA receptor antagonists only alleviate AD's behavioral and cognitive symptoms, but not its pathology (Cummings et al., 2019). As a result, searching for a disease-modifying therapeutic approach became an interest of many researchers. Cell-free (acellular) therapy for neurodegenerative diseases using exosomes is rising in popularity in recent years. In conditioned medium, Mesenchymal stem cells are known to secrete small biomoleclues known as secretomes. Examples of secretomes include lipids, nucleic acid, growth factors, cytokines and chemokines (L et al., 2019). Secretomes can be found freely in conditioned medium or carried by secreted vesicles from the cell known as extracelluar vesicle. Extracelluar vesicles are a group of membraned

vesciles packed with biomolecules, extracellualr vesciles are classified based on size into microvesicles and exosomes (Abels & Breakefield,2017). current approaches focuses on using smaller extracelluar vesicles (exosomes), as they can pass Blood Brain Barrier (BBB) (Banks et al., 2020.). mesenchymal stem cells derived exosomes (MSCs-exo) are considered a unique therapeutic approach of AD; as MSC-exosomes release paracrine factors that can ameliorate AD pathology and miRNAs, which can regulate glial and neural functions (Reza-Zaldivar et al., 2018).

1.2 Problem statement

Exosomes secreted from mesenchymal stem cells have been studied in various neurodegenerative disease models, including alzheimer's disease and traumatic brain injury (Ni et al., 2019;Bodart-Santos et al., 2019). However, there is a lack of evidence suggesting the role of MSC-exosomes in the treatment of AD as the characteristics and therapeutic mode of actions that MSC-exosomes can provide for AD treatment is not fully discovered. Therefore, there is a need for an empirical method to summarize study characteristics and results of MSC-exosomes effect on AD and assess the quality of the studies. In this project, a systematic review was used as the approach to unravel findings regarding the benefits of MSC-exosomes on AD models. Through this approach, it is anticipated that systematically convincing data from previous studies could be gathered, assessed and interpreted to provide more insights on the therapeutic potential of MSC-exosomes as the prospective therapy for AD.

1.3 Hypothesis

1. Exosomes derived from all mesenchymal stem cells sources provide essential content of exosomes with various efficiency for AD treatment
2. MSC-derived exosomes exhibit their therapeutic properties in treating AD through various mode of actions

1.4 Objectives

General Objective:

To collectively unravel the therapeutic potential of MSC- derived exosomes in treating AD through conducting a systematic review and analysis of the relevant works recently published from January 2016 until December 2020.

Specific Objective:

1. To find the sources of MSCs that are potentially essential to produce exosomes suitable for AD research.
2. To examine the characteristics and the contents of MSC-derived exosomes used in AD research.
3. To assess the therapeutic modes of action of MSC-exosomes as the potential source of treatment for AD.

CHAPTER 2

LITERATURE REVIEW

2.1 Alzheimer Disease (AD)

As of 2015, Over 46 million cases of Alzheimer Disease has been documented worldwide, and this number is expected to be over 131 million by the year 2050 (Prince et al., 2015). Based on National Health and Morbidity Survey of Malaysia (2018), prevelance of dementia was 8.5% in eldery people over the age of 60. Biggest challenges in AD include lack of definitive biomarkers for early detection, disease-modifying approaches and understanding of its pathophysiology pathways (Frozza et al., 2018)

2.1.1 Characteristics of Alzheimer's disease (AD)

2.1.1.1 Molecular characteristics

Two mains pathophysiology contribute to Alzheimer disease progression: 1) Amyloid Beta (Aβ) Plaque formation and 2) Phosphorlayted Tau (P-tau) protein formation. Aβ plaque are extracellular build up of two misfoldered amino acids Aβ42 and Aβ40, Aβ42 contributes to more insoluble and fibrillized monomores and its more common than Aβ40 monomers (Lane et al., 2018). The Aβ plaque formation starts with cleavage activity of the Amyloid precursor protein (APP). APP cleavage gives rise to two pathways: 1) non-amyloidogenic pathway, where APP is cleaved upon the combined action of two enzymes (α-secretase and γ-secretase) resulting in the secretion of the soluble forms of APP (membrane-bound C83 and soluble APP-alpha, sAPPα), and 2) the amyloidogenic pathway, where the APP fragment is cut by γ-secretase and β-secretase, subsequently leading to insoluble forms of APP membrane-

bound C99 and sAPPβ (**Figure 1**). The insoluble amyloid-beta monomers lead to the formation of amyloid plaques (Lopez et al., 2019).

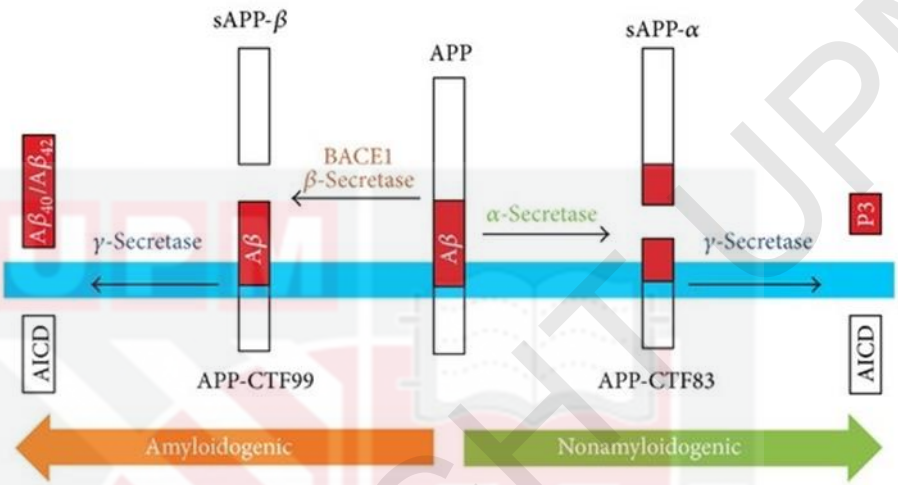

Figure 2-1- The amyloid precursor protein pathways (adapted from Pająk et al., 2016)

Tau protein is one of the noteworthy microtubules associated proteins (MAP) found in the cytoskeleton. Tau protein is mainly present in the brain and contributes to the stabilization of the cell cytoskeleton. Regulating the tau protein occurs by a balanced activity of kinases and phosphate in the amyloid cascade hypothesis (**Figure 2**). A disturbance of their activity's balance contributes to tau protein aggregation, subsequently leading to neurofibrillary tangles. (Jouanne et al., 2017).

Some studies suggest that mutation in specific genes influences the progression of AD; mutation in presenilin genes (*PSEN1* & *PSEN2*), apolipoprotein E (*ApoE*), and amyloid precursor proteins (*APP*) are among the molecular characteristics

found in AD (Lane et al.,2018). Familial Alzheimer's disease is caused by mutations in presenilin genes, while a mutation in *ApoE* causes sporadic AD.

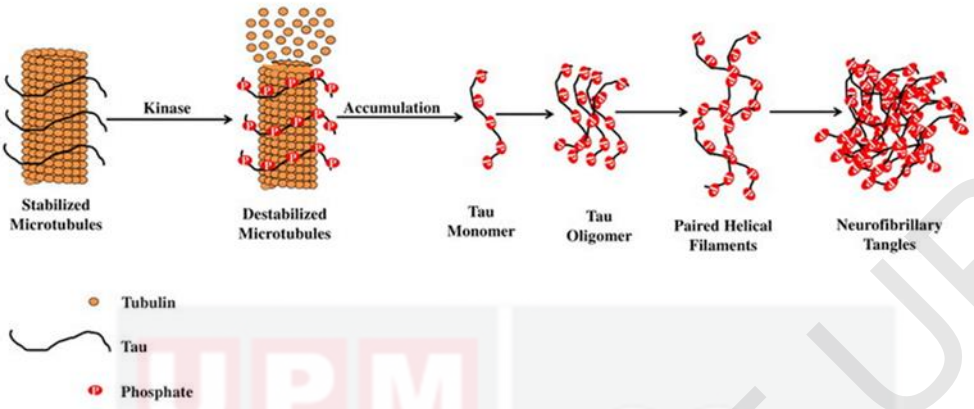

Figure 2-2- The tau protein pathology (adapted from Al Mamun et al., 2020).

2.1.1.2 Biochemical characteristics

Oxidative stress is one of the features of neurodegenerative diseases, including AD, and it is defined as the imbalance of oxidants and anti-oxidants towards the oxidants side. The formation of reactive oxidative species (ROS) is the hallmark of oxidative stress. ROS are highly reactive compounds generated in response to cellular or environmental factors; for example, exposure to smoking or air pollutants triggers ROS release. An example of ROS would be hydrogen peroxidase (H_2O_2) (Pallas & Camins, 2006). In the typical environment, there is a system called the anti-oxidant defence system. This system function to protect the lipid membrane, proteins, and cellular compartment from ROS by releasing anti-oxidants that counteract oxidative stress (Birben et al., 2012). Glutathione is an anti-oxidant that eliminates oxidants by donating one electron to ROS. Levels of glutathione decrease by age which explains why oxidative stress is a predominant trait of AD (Gella & Durany, 2009).

Glial cells are an integral cells that protects the central nervous system (CNS). Microglial and astrocytes, for example, have an essential role in protecting the brain in the early AD stages. However, upon the progression of the disease, glial cells activation might be a double-edged sword. Microglial activation has two types: classical activation (M1) and alternative activation (M2, **Figure 3**). M2 microglial activation occurs in early AD onset. In this activation, Microglia releases anti-inflammatory factors such as IL-10 and TGF-β that suppress the oxidative stress caused by amyloid plaques and neurofibilary tangles (NFT).

On the contrary, M1 microglial activation, which occurs in later stages of AD, releases pro-inflammatory factors such as Tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin (IL-12) that might damage the neurons instead of dealing with the trigger of its activation like amyloid plaque and neurofibilary tangles (NFT) (Kaur et al., 2019). In normal conditions, astrocytes work by clearing amyloid plaques via phagocytosis and through the secretion of degrading enzymes. When brains grow through injury or pathological circumstances, astrocytes become reactive and release pro-inflammatory factors including IL-17,and Interferon Gamma (IFN-γ) that disturb synapse and causes dystrophy of neurons (Garwood et al., 2011).

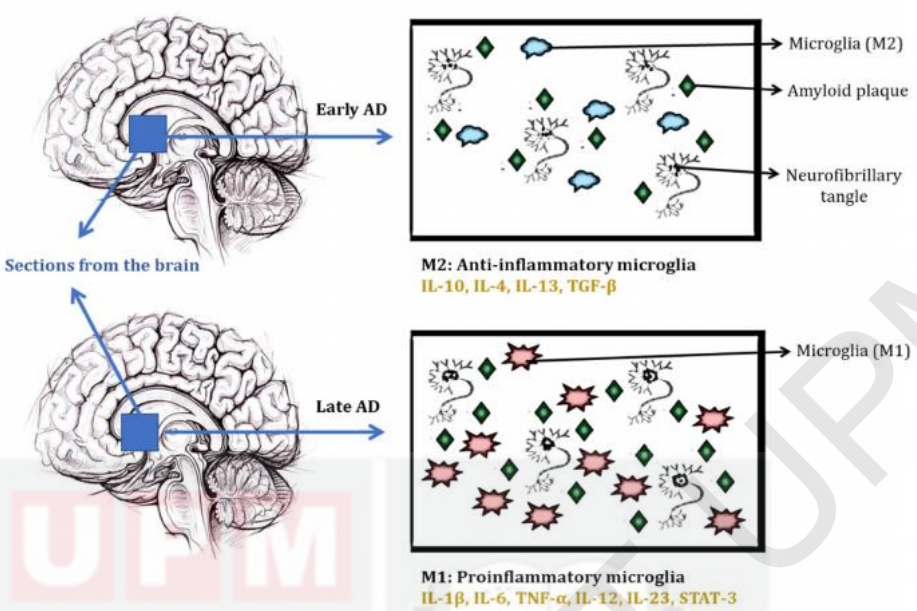

Figure 2-3 Types of microglial activation. Adopted from Kaur et al. Journal of Inflammopharmacology (2019).

2.1.1.3 Morphological characteristics and clinical presentations of AD

Due to the biochemical and molecular pathologies of AD, morphological changes and symptoms manifest. One of the pathophysiology seen in AD is synapse loss. Synapses are lost as amyloid plaques and tau proteins disturb the communications between neurons, causing brain atrophy. In AD, accumulation of Aβ oligomers affects synapse by the inhibition of long term potentiation (LTP), leading to the increase of long term depression (LTD) (Koffie et al., 2011). Morphological changes in AD include brain atrophy leading to widening of the ventricles and shrinkage of the gyrus (Khachaturian and Radebaugh, 1996, Bagad et al., 2013). Symptoms caused by AD vary based on the stage; in mild AD, symptoms include memory loss, longer time doing regular daily tasks, and repeating questions. Symptoms of the moderate stage are difficulty recognizing family and close friends, further confusion, and memory

loss. As for the severe stage, symptoms can go further to cause seizures and weight loss (Alzheimer's association, 2005).

2.1.2 Current treatment options for Alzheimer's disease

For AD, there are few therapeutic options: cholinesterase inhibitors and NMDA receptor antagonists. Another alternative treatment is a cholinesterase inhibitor and memantine combination. (Cummings et al., 2018). According to the cholinesterase hypothesis suggested by Perry (1986), AD contributes to the low activity of neurotransmitter acetylcholine (Ach) enzyme Acetylcholinesterase (AChE); leading to cognitive impairment. Cholinesterase inhibitors work by inhibiting acetylcholinesterases and butyrylcholinesterases, therefore preventing the breakdown of Ach (Hakansson, 1993). In AD, amyloid-beta plaques lead to excessive glutamate and NMD receptor activity, which triggers the alleviated influx of Ca2 into the cells, subsequently disturbing synaptic plasticity and detouring neurons. Therefore, introducing the NMD receptor antagonist drug as a managing approach prevents Ca2 influx, thus preventing cell death (Wang and Reddy, 2017).

2.2 Stem Cell

2.2.1 Introduction to stem cells

Cell therapy using stem cells is rising in popularity in recent years; stem cell therapy is considered a great utensil in treating chronic diseases where conventional treatment approaches are inconvenient. Stem cells are the undifferentiated cells found

within organisms through various life stages, including embryonic, fetal, and adult life stages (**Figure 4**). They give rise to the cells via a process known as differentiation, therefore helping in forming tissues and organs (Kolios & Moodley, 2013). There are two fundamental stem cell characteristics: self-renewal, where cells can have unlimited proliferation leading to the propagation of an indefinite number of cells, and potency as stem cells can differentiate and give rise to various cell types. (Biehl & Russell, 2009). Stem cells provide a diverse role in organ and tissue regeneration and can be categorized based on potency into four categories: unipotent, multipotent, pluripotent, and totipotent (Mahla, 2016)

2.2.2 Categories of stem cells

Stem cells are classified into two categories based on developmental stage: pluripotent stem cells (PSCs) and multipotent stem cells (MSCs) (**Figure 4**). PSCs are stem cells that can give rise to almost any cell type. An example of PSCs is embryonic stem cell (ESC) which is found in the inner cell mass of blastocytes (Ohnuki & Takahashi, 2015). Another example of PSCS is induced pluripotent stem cells (iPSCs); iPSCs are adult stem cells that undergo artificial modification to become a pluripotent stem cells, hence sharing similar characteristics with ESCs (Takahashi et al., 2007)

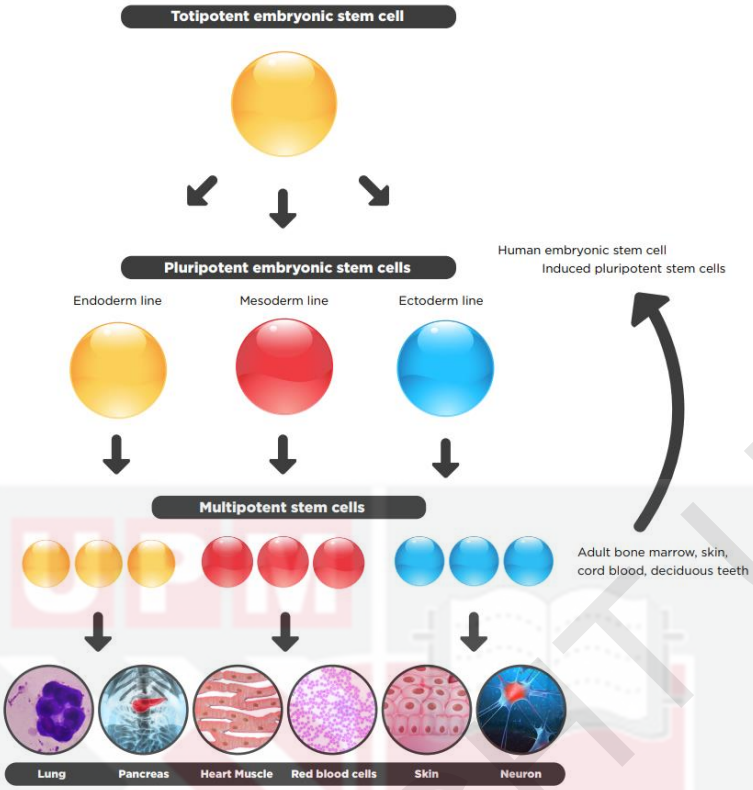

Figure 2-4 Types of stem cells based on potency (adopted from MacDonald 2018).

ESCs and iPSCs may give rise to ethical concerns as destroying embryos are required to obtain ESCs and safety issue in reprogramming procedure to generate iPSCs. Standardised good manufacturing practice (GMP) protocols are necessary to ensure iPSC's safety (Moradi et al., 2019). Therefore, multipotent stem cells (MSCs) come in handy as they devoid those issues. In addition to that, MSCs can be cultured into a specific lineage of interest, and they are safer as they don't form teratomas upon transplantation (Zomer et al., 2015). MSCs are stem cells that differentiate into numerous different cells but limited compared to PSCs. Examples of multipotent stem cells are adipose tissue stem cells and bone marrow stem cells. One of the multipotent stem cell trophic properties is immunomodulatory cytokines and growth factors (Aggarwal & Pittenger, 2005). In a review conducted by Mirzaei and fellow

researchers (2018), Multipotent stem cells were considered an attractive tool to use in clinical applications such as treating cancer, cardiovascular diseases (CVD), and inflammatory disease (Mirzaei et al., 2018).

2.2.3 Stem cell applications in therapy.

2.2.3.1 Cellular therapy

Multiple research of stem cell therapy has been noticed. The cellular therapy approach mainly uses stem cell transplantation as a mode of therapy. A study on spinal cord injury showed that ESCs could regenerate spinal cord tissue through transplantation of ESCs to the injury site (Shroff & Gupta, 2015). Induced pluripotent stem cell (iPSCs) was used to treat lung and liver disease by correcting Alpha-1 antitrypsin deficiency (A1AD) (Willson et al., 2015). Alpha-1 antitrypsin (AAT) is a protein that protects body tissue through the inhibition of neutrophil elastase enzyme (Strnad et al., 2020). Extensive cellular therapy research was conducted on MSCs. Bone marrow-derived stem cells (BMSCs) were used in blood clotting disorders, BMSCs were used to transplant megakaryocytes to produce more thrombocytes (Machlus & Italiano, 2013). Another use of MSCs was in Orodental abnormality; BMSC transplantation increases CD14+ & CD90+ levels, therefore, inducing jaw bone regeneration (Kaigler et al., 2013).

2.2.3.2 Acellular therapy

Cell-free (acellular) therapy utilizes the exogenous factors released from cells in the treatment of different diseases. Varying sources of mesenchymal stem cell-derived exosomes were used in acellular therapy research. Bone marrow-derived

exosomes (BMSCs), for instance, were used in the study of a rat model of liver fibrosis; the study indicated that bone marrow-derived exosomes reduced liver fibrosis in liver disease (Rong et al., 2019). As for adipose tissue-derived exosomes (ADSCs); various studies showed that ADSCs exosomes improved wound healing in a wounded mouse model by boosting fibroblast function (Zhang et al., 2018; Liu et al., 2019). Dental pulp stem cells (DPSCs) were also used in research. A study using a limb ischemia mouse model found that exosomes formed by dental pulp stem cells (DPSCs) can boost vasculogenesis (Iohara et al., 2008).

2.2.4 Secretomes of stem cell

Stem cell secretome is the general term that describes the secreted cell components that cause a paracrine modulatory effect (Xia et al., 2019). An example of secretomes would be cytokines, serum protein chemokines, and growth factors. Secretomes are released from the cells via extracellular vesicles (Abels & Breakefield, 2016). Extracellular vesicles were discovered over 70 years ago in 1946, when two scientists named Erwin Chargaff and Randolph Wes found that the extended centrifugation of blood plasma resulted in the loss of clotting factors, subsequently leading to a prolonged coagulation time. A follow-up study conducted in the late sixties identified vesicles derived from platelets as ‘platelet dust’ (Wolf, 1967). The terms exosome was first introduced by Tram and his colleagues in 1980. Recently, secretomes are used in cell-free therapy as they limit issues caused by cell therapy, such as tumorigenicity, in addition to providing therapeutic mechanisms by tissue repair and anti-apoptotic activity (Xia et al., 2019). Figure 5 shows the formation of different types of extracellular vesicles namely microvesicles and exosomes.

2.2.5 Content of stem cell secretomes

2.2.5.1 Microvesicles

Various extracellular vesicles analyses showed that microvesicles are released via the plasma membrane's direct outer budding (Minciacchi et al., 2015). They are more significant in comparison to exosomes as their sizes range between 100um to 1000um. Microvesicles contain phospholipids such as sphingolipids, phosphatidylserine, microRNA, proteins, and cell adhesion molecules such as integrin and selectin. It is also stated that more prominent extracellular vesicles, such as microvesicles, express CD40 (Ratajczak & Ratajczak, 2020). Microvesicles can be released from varying cells, including blood cells such as platelets, leukocytes, monocytes and neutrophils, microglia, dendritic cells, and adipocytes (Słomka et al., 2018).

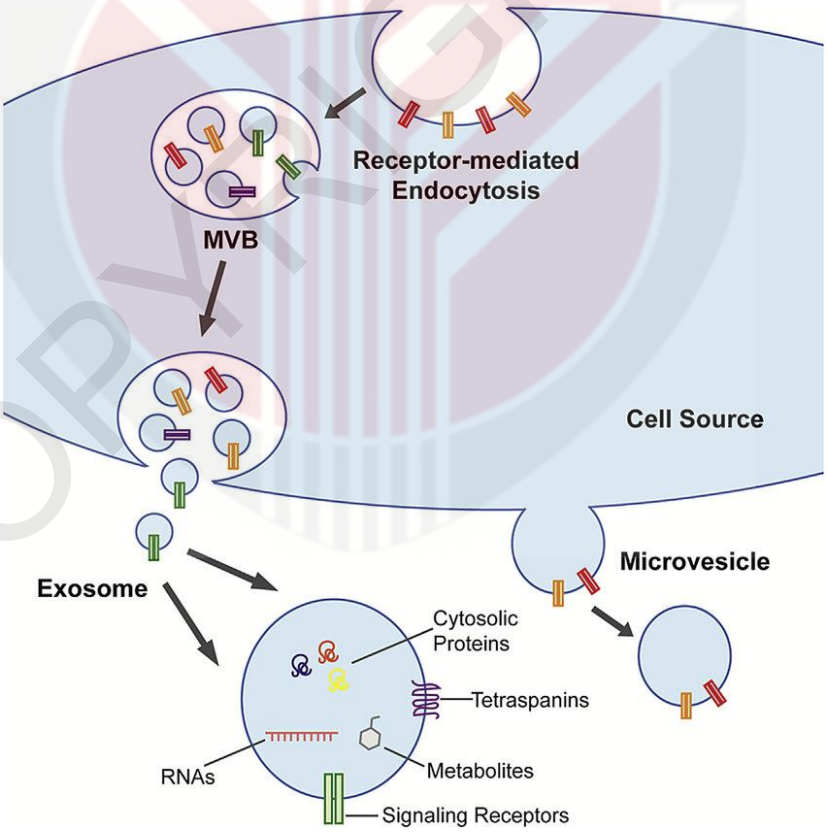

Figure 2-5 The formation of microvesicles and exosomes (adopted from Park et al., Progress in Retinal and Eye Research, 2017).

2.2.5.2 Exosomes

Exosomes are extracellular vesicles released by platelets, macrophages, mast cells, and reticulocytes, among other cells. (Denzer et al., 2000). Exosomes diameter range between 30nm and 150nm (Doyle & Wang., 2019). They are formed when the endosomal membrane inner budding fuses with the plasma membrane to create 'early endosome (EE)'. EE is further developed into the late endosome, then multivesicular bodies (MVBs), which contain intraluminal vesicles that release exosomes (Malm et al., 2016). Exosomes based tumour suppression strategy includes using it as a drug-delivery vector, where therapeutic microRNA and proteins are delivered to tumour cells or act as an anti-tumour vaccine to initiate immune response suppresses tumour progression (Dai et al., 2020).

Table 1 explains the types of extracellular vesicles. First type is exosomes,eosome diameter is usuakky below 150nm, it is originated from the endolysosomal pathway in cells, its content contains alix, tetrasapnins miRNA, proteins and lipids. Next are microvesciles, microvesicles range in size between 100-1000nm, they ate formed upon the cleavage of plasma membrane and consist of tetrasapnins , proteins and lipids. Lastly are apoptotic bodies, their diameter range between 50-5000nm, they are released in programmed cell death and contain cellular debris, apoptotic markers and proteins.

Table 2-1-Types of Extracellular vesicles (Butler et al., 2018)

EV	Size (nm)	biogenesis	composition
Exosomes	Below 150 nm	Endolysosomal pathway in cells	Alix, tetrasapnins miRNA, proteins, lipids.
Microvesicles	100-1,000 nm	Cleavage of plasma membrane	Tetrasapnins , proteins, lipids.
Apoptotic bodies	50-5,000 nm	Released in programmed cell death	Cellular debris apoptotic markers, proteins.

2.2.5.3 Isolation and Characterisation of Exosomes

Exosomes can be extracted from biological body fluids (e.g., cerebrospinal fluid, urine, saliva, and blood plasma). Exosomes can also be isolated from mesenchymal stem cells as well, and they facilitate intercellular communication (Rani et al., 2015) . Methods used for the isolation of exosomes depend on either particles' size or affinity (Zhu et al., 2020). Standard isolation methods include ultracentrifugation (dUC), immunoaffinity, and poly-ethylene glycol (PEG) based precipitation density (Sidhom et al., 2020). Characterization of exosomes is conducted to determine the physicochemical properties of exosomes, hence determining their biological interactions. The standard methods used for reporting exosomes properties are Western blot, nanoparticle tracking analysis (NTA), and flow cytometry

(Gurunathan et al., 2019). . Figure 6 shows commonly used isolation and characterization methods for exosomes.

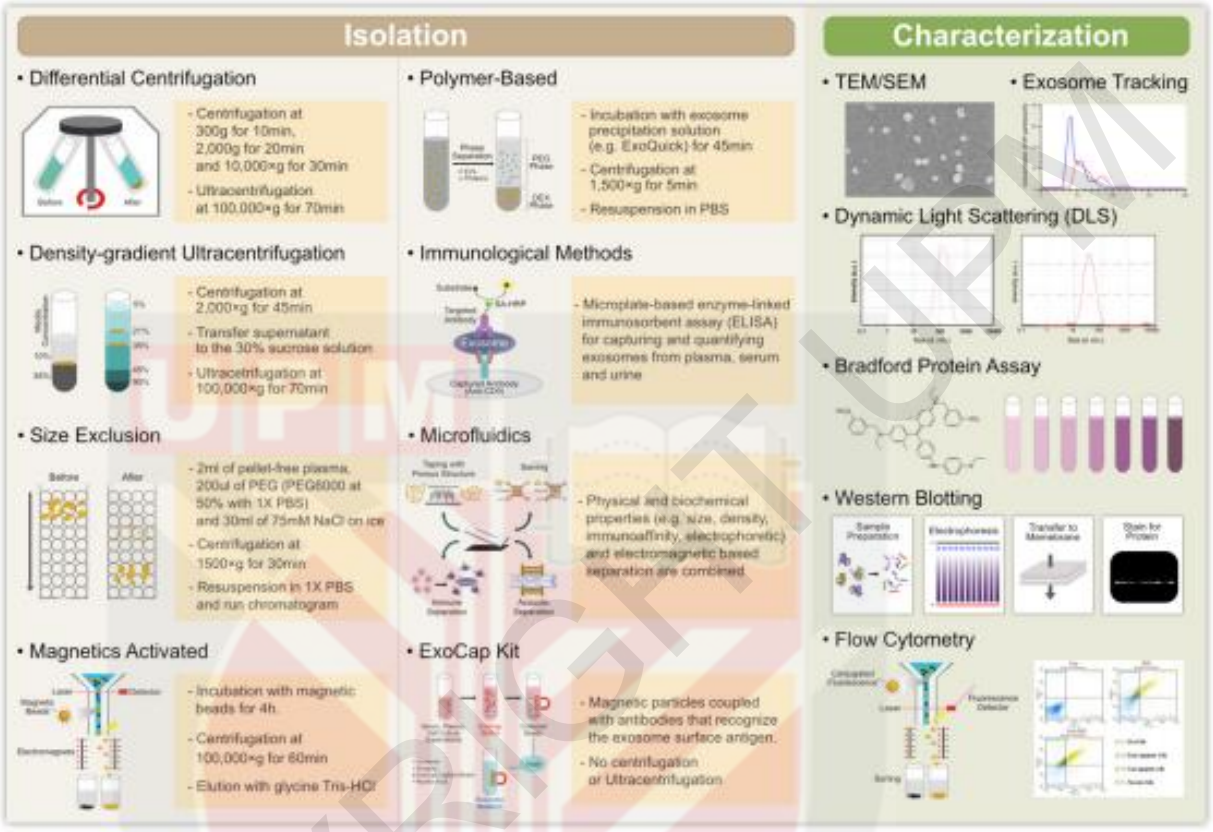

Figure 2-6- Methods of isolation and characterisation of exosomes (Gurunathan et al., 2019). Centrifugation bases isolation include differential centrifugation and density-gradent ultracentrifugation. In these methods, exosomes are obtained through the high-speed spinning of sample. Characterization methods include western blot, western blot reveals the expression of exosomal markers. Transmission electron microscopy (TEM) is a characterization method that visualise exosomes

Exosomes contain membrane-associated proteins called tetraspanins (e.g., CD81, and CD9, CD63). Tetraspanins play a role in the formation of exosomes and extracellular vesicles and the uptake of exosomes into recipient cells (Jankovičová et al., 2020). Exosomes also contain GTPases hydrolases enzymes eEF1A1 and eEF2, which are involved in the translation phase in ribosomes (Atkinson, 2015). They also contain heat-shock proteins (e.g., Hsp90 and Hsp60), which are thought to be involved in intercellular communications (Reddy et al., 2018). Exosomes also express Alix and

TSG101, a cell-surface proteins involved in the formation of MVB. in addition to lipid raft components (e.g., sphingolipid.), and miRNA.

2.2.6 Sources of stem cells and their properties

2.2.6.1 Adipose-derived Stem cell (ADSC)

Exosomes derived from adipose stem cells are thought to control biological processes; they start immunomodulation activity in type M2 macrophages by expressing IL-10 and Arg-1 in cultured obese mouse ADSCs (Zhao et al., 2018). Ni et al. (2018) identified over 1460 proteins relevant to cell signalling pathways and function through proteomic analysis.

2.2.6.2 Bone marrow-derived stem cell (BMSC)

Recently, bone marrow stem cell was proven to release exosomes that influence bone remodelling, destruction, and generation by regulating osteoclasts and osteoblast activities (Lyu et al., 2020). A study conducted by Ge et al. (2017) indicated the presence of over 1500 proteins, including proteins involved in the pathway of osteoblast, such as transforming growth factor-beta receptor 3 (TGFBR3) and bone morphogenetic proteins (BMP). They concluded that osteoblast derived exosome could provide insights in bone disease research.

2.2.6.3 Dental pulp derived stem cell (DPSC)

Dental pulp stem cell-derived exosomes have been the subject of interest in the regenerative medicine field. Like other mesenchymal stem cells, DPSCs possess therapeutic properties. One study used DPSCs to treat muscular dystrophy in a dog, the outcome of the study revealed notable engrafment of DPSCs cells in dog muscles (Kerkis et al., 2009). Based on a study conducted by Alkhayal et al. (2021), the

proteomic analysis found approximatally 1500 proteins about cells function and pathways.

2.2.6.4 Human umbilical cord-derived stem cell (HUCMSC)

Human umbilical cord stem cells are an appealing source of stem cells; the collection procedure of HUCMSC is less invasive than BMSCs as this discarded fetal tissue is targeted for the collection of cells (Ding et al., 2015). In a recent study using Human umblilcal cord stem cells extracellular vesicles (HUCSC-EV) to treat chronic obstructive pulmonary disease (COPD), HUCSC-EV exerted an anti-inflammatory effect through altering the expression of p65; P65 is a subunit of the pro-inflammatory factor NF-κB.

2.3 The future direction of stem cell-exosomes in treatment of AD

2.3.1 Neuroprotection for AD

Exosomes generated from mesenchymal stem cells have been suggested as a treatment for neurodegenerative diseases in multiple studies. In Parkinson's disease, a study showed that MSC-exosomes improved neural functions and stimulated the generation of oligodendrocytes (Xin et al.,2017). While for multiple sclerosis, MSC-exosomes have neuroprotective property through the secretion of anti-inflammatory factors such as mi146a and neuroprotective component such as Phosphatidylserine (Riazifar et al., 2019)

As for the current treatment of AD, it only works by managing the symptoms of the disease. mesenchymal Stem cells were proposed to provide alternative therapeutic approaches. Multiple studies suggested that AD can be treated via the

degradation of Aβ plaque via the act of degrading enzymes (Elia et al., 2019, Ahmed et al., 2016). In a review conducted by Hosseini et al. (2018), immunomodulation of microglial activity was introduced as a neuroprotection therapeutic approach; neuroprotection was observed through the regulation of apoptotic gene activity by reducing pro-apoptotic genes such as *PTEN* and *Fas* (Matsuda et al., 2018).

2.3.2 Development of in-vitro and in-vivo AD model

Current preclinical studies on AD are using in-vivo and in-vitro animal models to assess exosomes effects on AD. Transgenic animal models are genetically altered animals to study the function of the desired gene fragment. The widely accepted method of creating a transgenic mouse is through microinjection of desired DNA fragment into pronuclei of fertilized mouse embryos and inserting it into the oviduct of a pseudo-pregnant mouse; so it further carries the transgene (Kumar et al.,2009). As for the in-vivo approach, various mouse models are used, including single transgenic mouse models such as Tg2576 which only express the swedish amyloid preceursor protein ($A\beta PP_{swe}$). Double transgenic mouse models for AD express mutations in two genes which are *APP* and *PS1*. 5xFAD mouse model express swedish amyloid preceursor protein mutation (*AβPPswe*), indiana mutation ($A\beta PP_{ind}$), and florida mutation ($A\beta PP_{flo}$) and *PS1*. (Esquerda-Canals et al., 2017). The most common type used for AD research is APP/PS1 mouse models, bearing the mutant APP_{swe} and the mutant *PS1* genes (Jankowsky et al., 2004).

Although in-vitro AD models are less representative of AD pathology than in-vivo models, some research utilized the in-vitro model to study MSC-exosomes efficacy. SH-SY5Y Human neuroblastoma cells have been investigated intensively to

provide a model of neurodegenerative diseases. A study conducted by Arrozi et al (2017) evaluate the expression of Aβ40/42 ratio in cells transfected with SH-SY5Y. Cells were transfected with APPswe/ind gene using Lipofectamine 3000 transfecting reagent and pcDNA3-eGFP plasmid. By optimizing transfecting reagent, plasmid concentrations and other factors like incubation time and cell density, SH-SY5Y cells showed a transfection level of 40% and a high Aβ40/42 ratio, making it an applicable candidate for drug testing. Figure 6 demonstrate transgenic mice formation process

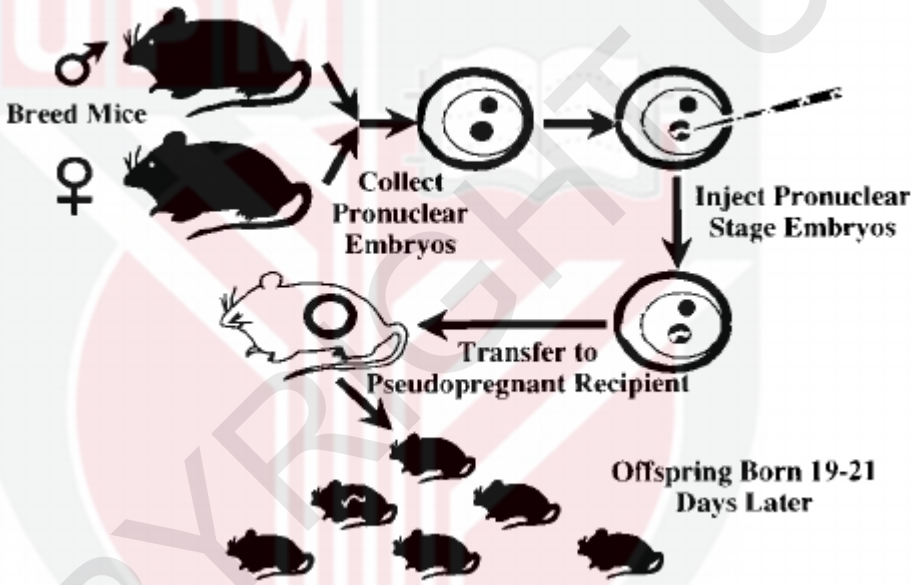

Figure 2-7 The process of forming transgenic mice via microinjection of the desired fragment. (Adopted from Roths et al. Journal or Comparative Medicine, 1999). Firstly, proneuclear embryos are collected from the breeding mice. After that, desired fragment is injected into the pronuclear. Lastly, the transformed pronuclear is transferred into a pseudopregnant recipient to give birth to transgenic offsprings.

2.3.3 Molecular biomarkers for the treatment of AD

A biomarker refers to a naturally occurring biological or molecular substance whereby physiological or pathological processes are assessed and evaluated based on their characteristics (Oyejide et al., 2017). An example of a molecular biomarker in AD is Beta secretase-1 (BACE1) which is involved in the formation of plaques. Microtubule-associated protein 2 (MAP2) and Doublecortin (DCX) are two proteins involved in microtubules stabilization (Gleeson et al., 1999). Markers involved in synaptic plasticity include postsynaptic density protein 95 (PSD-95), a post-synaptic Scaffolding protein, and Synaptophysin, a pre-synaptic protein that regulates synaptic vesicles (Chen et al., 2011).

Synaptogenesis and neural migration can be evaluated by polysialylated-neural cell adhesion molecule (PSA-NCAM) levels (Quartu et al., 2008). During neural damage or injury, glial fibrillary acidic protein (GFAP) expression is considered a hallmark for astrological activation (Glushakova et al., 2018). Microglial activations have a different marker determining the type of activation that occurred; for example, an ionized calcium-binding adaptor molecule (Iba1) is used as a marker for classical activation (M1) of microglia, while Chitinase-3-Like-3 (Chi3l3/Ym1), Arginase 1 (Arg1), and Resistin-like molecule alpha (FIZZ1) expression are increased in alternative activation (M2) (Tang & Le, 2016).

2.3.4 MSC derived-exosomal miRNA for AD treatment

Mesenchymal stem cell-derived exosomes (MSC-exo) transfer their content towards nearby cells, one of the components transferred are MicroRNAs (miRNA). miRNAs are an attractive tool for the diagnosis and treatment of serval pathologies (Asgarpour et al., 2020). Potential rules of miRNA in the treatment of Azheimer's Diseases (AD) was revealed in a review conducted by Reza-Zaldivar et al (2018), It is thought that miRNA adjust gilal and neural cells activity as over 70% of miRNAs are expressed in the brain, and exosomes contains more miRNA than its origin cells. As for miRNA effects in AD treatment, a study revealed that miR-29c downregulation contribute for the escalated expression of beta secretase (BACE1) enzyme in AD (Lei et al., 2015). Another study revealed reduction in Aβ plaque formation as miR-340 targetted BACE1 (Tan et al., 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Flowchart of methodological procedure

Figure 8 describes the methodology process. First, the research question was constructed where the research gap was addressed; then, research protocol was developed by following PRISMA protocol steps, defining keywords, eligibility criteria, and choose databases. Next, data is extracted from research articles, including information regarding MSC-exo, the population used, and study outcome. Lastly, the risk of publication bias is performed to assess the internal validity of data.

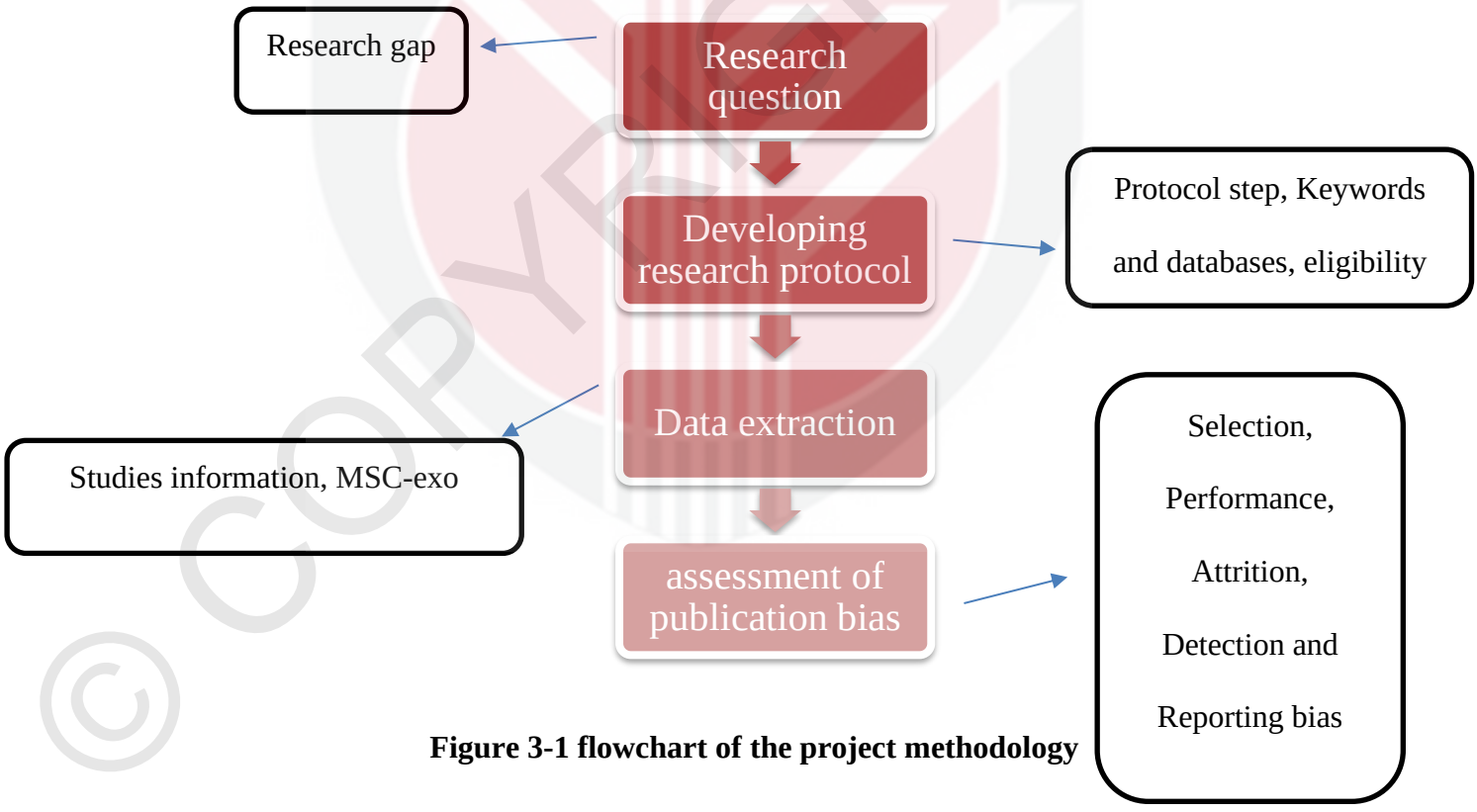

Figure 3-1 flowchart of the project methodology

3.2 Formulating a Research question

There are ongoing trials on mesenchymal stem cell-derived extracellular vesicles and exosomes being a candidate for AD treatment. However, as of writing this study, no systematic review has thoroughly elucidated the ongoing research on mesenchymal derived exosomes in AD therapy. Therefore, this study aims to fill the gap and provide a bridge to gather and assess existing information. First, a research question was developed by defining the Population (P), Intervention (I), Comparison (C), and Outcome (O) by referring to the PICO principle created by Ahn & Kang (2018). The formed question was: Does MSC-Derived stem cell exosome (I) ameliorate AD Pathology (O) in comparison to control group (C) in AD models (P)?

3.3 Developing a Research Protocol

The search protocol used was Preferred Reporting Items for conducting Systematic Reviews and Meta-analysis (PRISMA), which contains a flowchart of four phases: identification, screening, eligibility, and included studies (Moher et al., 2009). Before initiating the search, keywords, database, and eligibility criteria were predefined.

3.3.1 Steps of PRISMA protocol

Figure 9 explains the PRISMA protocol in a flowchart. The flowchart consists of four steps: identification, screening, eligibility, and inclusion. In the identification step, studies are identified in the chosen databases using a constructed keyword. Then the duplicates found within databases are removed. In the screening step, records are screened based on abstracts and excluded based on defined eligibility criteria. In the eligibility step, the remaining records are assessed by reading the full article text. Studies that do not fulfil the predefined eligibility criteria are excluded. The remaining research studies that fit the requirements are included in the qualitative assessment in the last step. The data were extracted from the research result and incorporated in the evaluation of publication bias.

3.3.2 Keywords and Databases

Before initiating the search through the databases, keywords were selected. as for keywords, six keywords were selected: AD, Secretome, Exosome, Extracellular vesicle, Acellular therapy, and Cell-free therapy. The method of using keywords for the search on the different databases was: “Alzheimer’s diseases” AND “Secretome”, “Alzheimer’s diseases” AND “Exosome”, “Alzheimer’s diseases” AND “Extracellular vesicle”, “Alzheimer’s diseases” AND “Acellular therapy”, “Alzheimer’s diseases” AND “Cell-free therapy”. Different keywords combinations were used to find additional relevant research papers from all databases selected. Research studies relevant to mesenchymal stem cell and Alzheimer diseases were obtained from PubMed, Scopus, and Ovid Medline. Multiple Databases wre used to find more extensive researches.

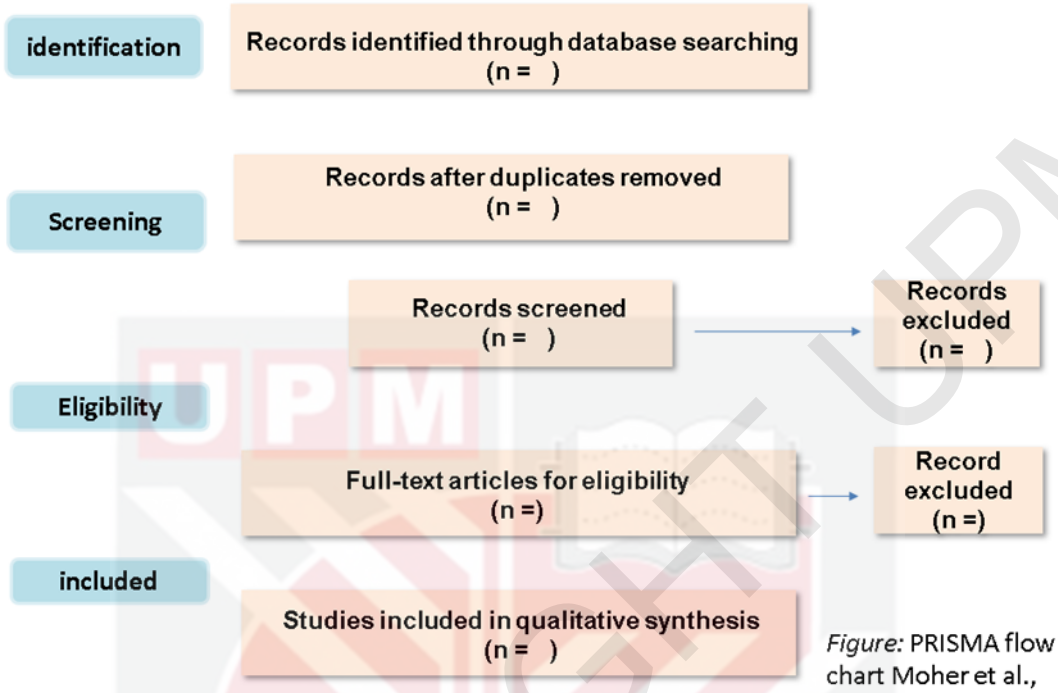

Figure: PRISMA flow chart Moher et al., (2009)

Figure 3-2- PRISMA flow chart

3.3.3 Eligibility criteria

3.3.3.1 Inclusion criteria

Literatures that fulfilled the criteria mentioned below were selected for inclusion in this study:

A. Studies on mesenchymal stem cells (MSCs) derived- exosomes therapeutic effect on Alzheimer' Disease (AD)

Our main objective is to provide evidence on MSC-exo therapeutic effect on AD; therefore these studies were included.

B. Studies conducted between Jan 2016 –Dec 2020

To search for novel recent studies, the timeframe for the search was set to last five years. As the study started on Oct 2020, Dec 2020 was selected to be the last date of publication in order to proceed with analysis.

C. Original research papers

This study aims to assess ongoing original research paperwork as they provide empirical evidence on Mesenchymal Stem Cells derived- exosomes **(**MSC-exo) effect on AD.

D. Interventional studies

Research ongoing on MSC-exo effect on AD uses MSC-exo as an intervention was selected for the review.

E. Studies in English language

To make our finding globally accessible, this review only focused on studies conducted in English language.

F. In-vivo and in-vitro study designs

To provide evidence using multiple study designs, we used findings conducted in-vitro and in-vivo.

G. Animal and human studies

To provide evidence using various study population, studies including animal and human populations were included.

3.3.3.2 Exclusion criteria

For exclusion criteria in this review, Literature that met the criteria listed below were excluded:

A. Studies on other neurodegenerative diseases (e.g., Parkinson disease)

The objective of this study is limited to Alzheimer disease therefore, other studies were excluded

B. Studies conducted outside the timeframe listed in inclusion criteria

The timeframe listed for the study is Jan 2016-Dec 2020. Studies conducted before Jan 2016 were excluded as recent novel studies are required. Studies conducted after Dec 2020 was excluded from proceeding with data extraction and publication bias assessment

C. Review papers and commentaries

Review papers and commentaries were excluded as they do not provide empirical evidence on MSC-exo effect on AD

D. Non-interventional study

Not many other study designs were established as most of the research on MSC-exo on AD were pre-clinical studies; therefore non-interventional studies were excluded.

E. Studies in other languages

Studies in other languages might affect the process of study as the translation process might be lengthy, and translators for other languages are needed. Hence, publications in other language than English were excluded

F. Exosomes derived from other types of stem cells (e.g., iPSCs and ESCs)

Since this study focused on MSCs derived exosomes, studies of exosomes derived from other types of stem cells were excluded.

3.3.4 Data extraction

After defining studies for qualitative analysis, the following information was retrieved from all the studies: author, year of publication, characteristics of in-vivo and in-vitro AD model used, route of exosome/extracellular vesicles administration. For the in-vivo AD model, behavioral tests and immunohistochemical tests were conducted. Details regarding MSC-exosome properties were collected, including types of stem cells, exosome characterization, isolation performed, and exosome size distribution. Information regarding the following MSC exosomes therapeutic neuroprotective mode of actions effects was collected: degradation of Aβ plaques, amelioration of morphological deterioration, cell migration and apoptosis, microRNA and markers expressions, inflammatory assessment such as the assessment of inflammatory cytokines levels, microglial and astrocytes activation. Other data extracted were duration of treatment induction and type of culture plate.

3.3.5 Assessment of publication bias

Studies related to MSC-exosomes were assessed for internal validity using the Risk of Bias (RoB) assessment tool developed by Office of Health Assessment and Translation (OHAT). The following types of biases were assessed:

A. Selection bias:

This bias assesses is the differences in baseline characteristics between intervention and control that indicates how study subjects are allocated to each group.

Domains addressed in this bias are:

i) random sequence generation

ii) allocation concealment

B. Performance bias:

This bias addresses the groups' differences in handling or exposure to external factors affecting the results. (e.g non-random housing of animals to get desired results)

Domains addressed in this bias are:

i) Identical experimental conditions

ii) Blinding of researchers during the study

C. Attrition bias:

Attrition bias describes the difference between groups due to possible loss of participants

Domains addressed in this bias are:

i) missing data outcome

D. Detection bias:

Detection bias refers to differences in the outcome measures of groups due to how these outcomes were assessed.

Domains addressed in this bias are:

i) Exposure characterization

ii) Outcome assessment

iii) Blinding of outcome assessors

E. Reporting bias.

Lastly is reporting bias, which refers to the variation in study outcome due to selective reporting of study outcome.

Domains addressed in this bias are:

i) Outcome reporting

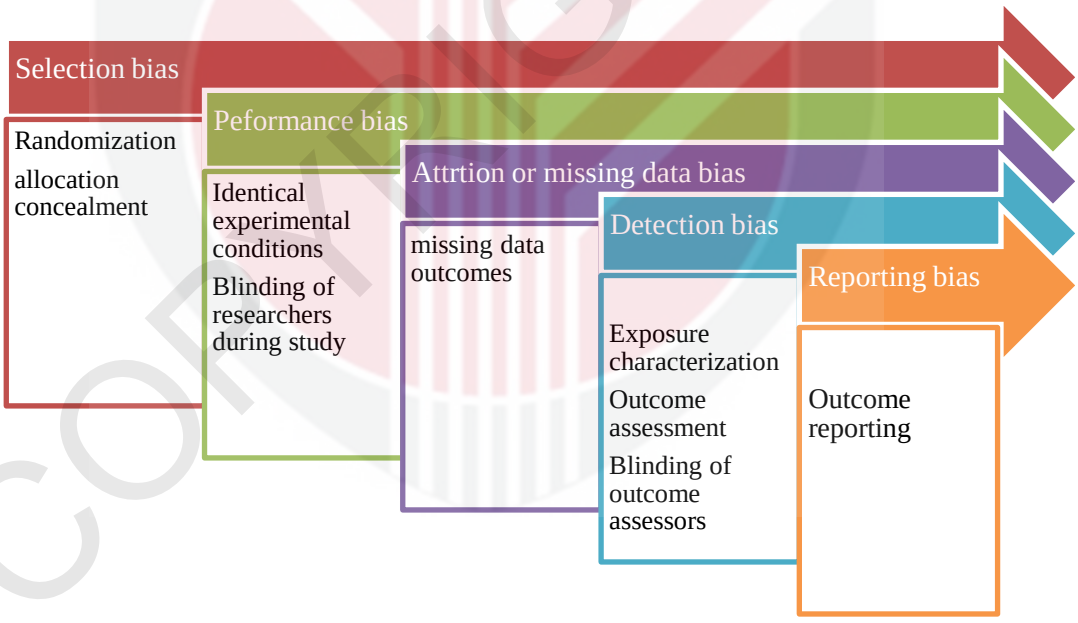

Figure 3-3 Biases and domains assessed in OHAT risk of bias assessment tool

Table 2 shows the key figure for each level of biases; biases are categorised into four levels:

A. Definitive low risk of bias

In this category, there is a direct proof for low risk of bias; an example of the low-risk practice might be included.

B. Potentially low risk of bias

There is indirect proof for low risk of bias in this category, or it seems that the possible practice won't result in bias.

C. Potentially a high risk of bias

There is indirect proof for the high risk of bias in this category, or no suffitient information has been provided regarding the possible bias.

D. Definitive high risk of bias

In this category, there is direct proof for high risk of bias, an example of the high risk practice might be included

Table 3-1- Key figures of risk of bias levels

++	Definitive Low risk
+	Probably low risk
- NR	Probably High risk
--	Definitive high risk

CHAPTER 4

RESULTS

4.1 Search Results

Figure 11 demonstrates the systematic search process, and figure 12 shows the number of records identified according to the keywords. In the identification step, a total of 3178 records was found through the following selected databases: PubMed, Scopus, and Medline. 251 record remained upon duplicate exclusion. Records were then subjected to first screening according to pre-defined eligibility criteria, and 232 records were excluded, with 19 remaining. Some of the excluded studies were review papers, papers conducted on other Stem Cells than MSC-exo, and papers conducted outside the selected timeframe 'Jan 2016 –Dec 2020'. 19 remaining records were subjected to full text screening for eligibility. 11 records remained while 4 review and commentaries papers, 3 papers on other types of stem cells and one study not related to MSC-exo were excluded.

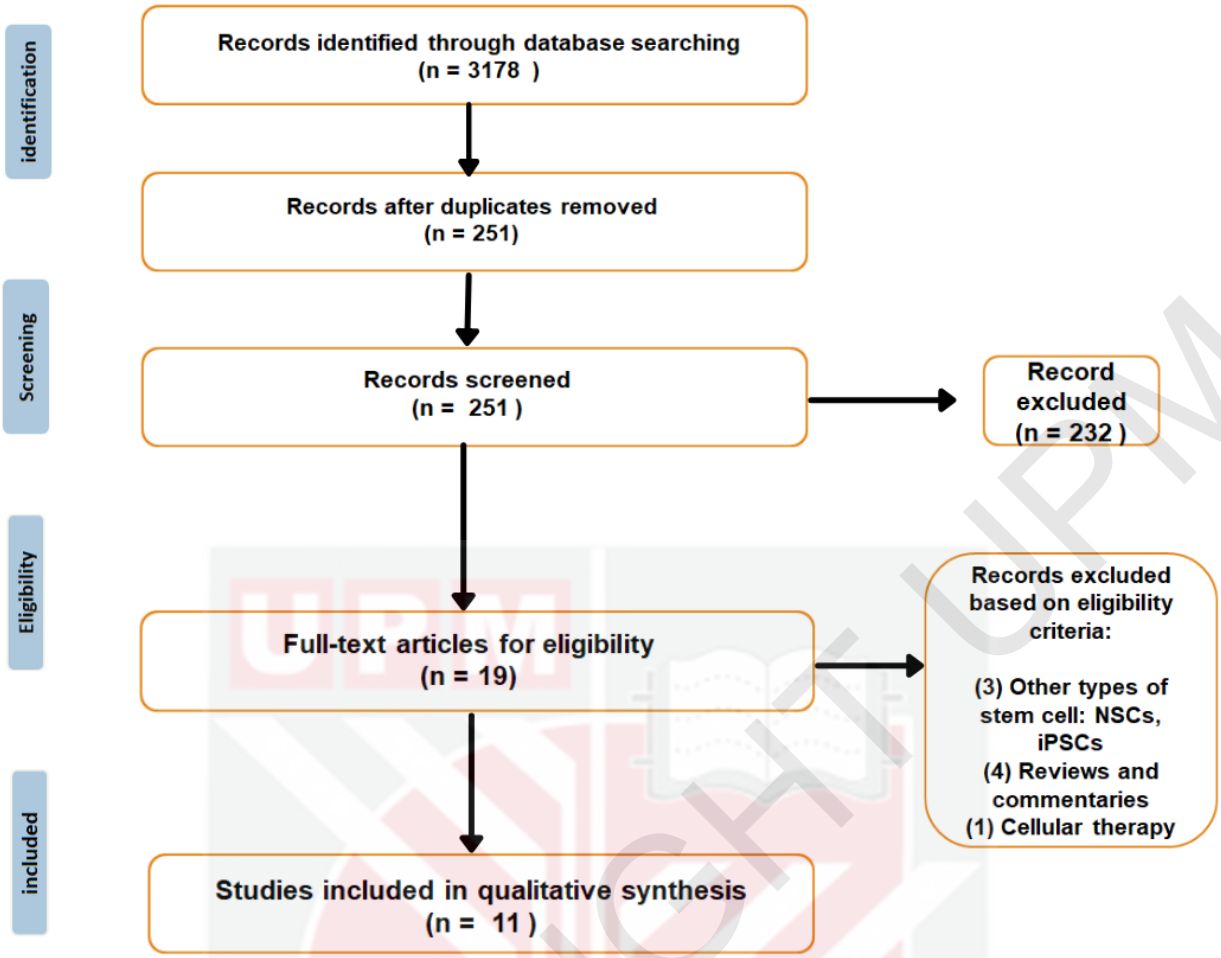

Figure 4-1 Result for flowchart search process following PRISMA guidelines

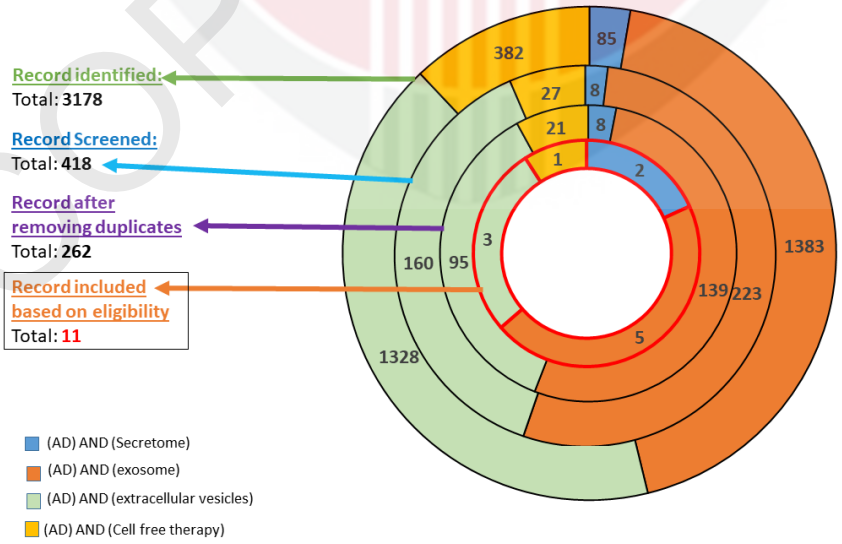

Figure 4-2 Record found through databases using keywords

4.2 Description of studies

Overall, 11 research articles were selected for qualitative analysis. The articles were published from January 2016 to December 2020. Most of the identified research articles were published in 2020, accounting for 36.36% of the total records found. 2016 had the least records with approx 9% of total records (Table 2).

As for sources of MSC exosomes/EV, multiple sources were identified including five from BMSCs (45.45%), two from ADSCs (2/11,18.18%), two from HUCSCs (2/11,18.18%), one from DPSCs (1/11, 9.09%) and one was unspecified (1/11, 9.09%). five studies in-vivo studies used APP/PS1 mouse animal models as their test subjects (5/11, 45.45%), while only one study used C57BL/6 with Aβ aggregates (1/11, 9.09%).

Various route of exosomes/EV administrations were identified including Intercerebral injection (ICV) (3/11, 27.27%), Intravenous (IV) (2/11, 18.18%) and intranasal (1/11, 9.09%). For in-vitro studies, different cells were used as test groups, including SH-SY5Y Cells (2/11, 18.18%) and neurons treated with Aβ aggregates (3/11, 27.27%). Other types of cells used include neurons from transgenic animal (2/11, 18.18%), BV2 cells (1/11, 9.09%) and astrocytes (1/11, 9.09%) (Table 3).

Age of animal varied between 7-8 weeks up to 13 months. As for the duration of incubation, it differed between 4hrs to 72hrs.

Table 4-1- Year of publication for the studies included

Year of publication			
2016	1	11	9.09%
2017	2	11	18.18%

2018	2	11	18.18%
2019	2	11	18.18%
2020	4	11	36.36%
Total number of papers	11		

Table 2-2 Characteristics of included studies categorized based on: Source of MSC exosomes/EV, Route of administration, in-vivo and in-vitro AD models

Category	Number of studies	Percentage
Source of MSC exosomes/EV		
BMSCs	5	45.45%
ADSCs	2	18.18%
HUCSCs	2	18.18%
DPSCs	1	9.09%
Not specified	1	9.09%
Total number of studies	11	
Route of Administration (for in-vivo studies)		
ICV	3	27.27%
IV	2	18.18%
Intranasal	1	9.09%
Total number of studies	**11**	
In-vitro AD model		

SH-SY5Y Cells	2	18.18%
Neurons treated with Aβ aggregates	3	27.27%
Neurons from transgenic animal	2	18.18%
BV2 cells	1	9.09%
astrocytes	1	9.09%
Total number of studies	**8**	
In-vivo animal model		
Mouse-APP/PS1	5	45.45%
Mouse-C57BL/6 with Aβ aggregates	1	9.09%
Total number of studies	**6**	
Age of animal model (in-vivo studies)		
7-8 weeks	1	9.09%
3-5 months	1	9.09%
7 months	1	9.09%
10-13 months	1	9.09%
Not specified	2	18.18%
Total number of studies	**6**	
Duration of cell incubation (in-vitro studies)		

4 hours	2	
12 hours	1	
22 hours	1	
24 hours	2	
48 hours	1	
72 hours	1	
Total number of studies	**8**	

4.3 Formulation of research question

Based on PICO principle, summary of all studies were evaluated in order to formulate relevant research question before addressing the objection of this systematic review. Table 4 describes the summary of the 11 studies reviewed. In general, the studies revealed the therapeutic potential of MSC-derived exosomes for treatment od AD through various mode of actions. The exosomes were discovered to posses neuroprotective properties by degrading or reducing deposition of Aβ, regulating apoptosis, regulating inflammatory cytokines and microglial activation levels, regulating inflammation through the uptake of exosomal miRNAs and increasing neurogenesis. Table 4 comprehensively describes the summary of the 11 studies reviewed based on PICO principle.

4.4 Summary of all study objectives and outcomes

Table 0 elaborate on the objectives and outcomes of the 11 included studies. All objectives aimed to investigate how mesenchymal stem cell-derived exosomes (MSC-Exo) mechanisims in treating Alzheimer's Disease (AD) by mechanisim that might reduce cytotoxicity, ameliorate AD pathology, improve cognitive impairment and reducing AD burden. The outcomes of the studies revealed various moode of action of MSC-Exo which include reducing amyloid beta (Aβ) plaque by the act of degrading enzymes, decline of oxidative stress, regulating apoptosis, delivering various miRNAs, decreasing neuroinflammation by targeting gilal cells or pro-inflammatory factors and enhancing neurogenesis.

Table 4-3 Summary of all studies outcomes

Author and year	Type of study	population	intervention	objective	Study outcomes
Ahmed et al. (2016)	In-vitro	SH-SY5Y Cells	DPSCs-secretomes	To find the therapeutic potential of secretome derived from DPSCs in reducing cytotoxicity and apoptosis caused by amyloid-beta (Aβ)	DPSCs secretome has neuroprotective properties by degrading Aβ, regulating apoptosis.
Lee et al. (2018)	In-vitro	Neuron from transgenic mice	ADSCs-exo	To investigate modifying effects of ADSCs exosomes in ameliorating Aβ induced AD Pathology	ADSCs-exo has neuroprotective properties by degrading Aβ and regulating apoptosis.
De Godoy et al. (2017)	In-vitro	Neuron treated with Aβ aggregates	BMSCs-EV	to evaluate the neuroprotective potential of mesenchymal stem cells (MSCs) against AβOs derived oxidative stresss on hippocampal neurons	BMSCs-EV has neuroprotective properties by reducing oxidative stress
Wei et al. (2020)	In-vitro	SH-SY5Y cells	HUCMS-exo	To explore whether MSC-derived exosomes have protective effects on neuronal apoptosis and whether this effect is miR-223 content dependent.	HUCMS-exo has neuroprotective properties by regulating apoptotic and inflammatory cytokines levels

Nanako et al (2020)	In-vitro	-Astrocytes	BMSCs-exo	To investigate whether BM-MSCs can improve cognitive impairment in AD model mice and how they repair damaged astrocytes, focusing on miR-146a.	BMSCs-exo has neuroprotective effect by reducing inflammatory markers through the uptake of exosomal miRNA-146a by astrocytes
Ding et al. (2018)	In-vivo,in-vitro	-Mouse-APP/PS1 -BV2 Cells	HUCSC- exo	To investigate the effect of HUCSC-exos on neuroinflammation, microglial reaction, Aβ deposition, and cognitive functions of AD mouse model and BV2 cells	HUCSC-exosomes have a therapeutic effect on by reducing Aβ plaque deposition and inflammatory microglial activation and induce alternative microglial activation in both in-vivo and in-vitro
Ma et al. (2020)	In-vivo,in-vitro	-Mouse-APP/PS1, -Neuron treated with Aβ aggregates	ADSCs-exo	To explore the effect and mechanisms of ADSC-exos on ameliorating neuronal damage	ADSCs-exo has neuroprotective properties by degrading Aβ in-vivo /in-vitro and regulating inflammatory microglial expression in-vivo
Wang et al. (2017)	In-vivo,in-vitro	Mouse-APP/PS1 -Neuron from transgenic mice - Neuron treated with Aβ aggregates	BMSCs-exo	To investigate the effect of MSC-derived exo on ameliorating oxidative stress	BMSCs-exo has neuroprotective properties by degrading Aβ.
Elia et al. (2019)	In-vivo	Mouse-APP/PS1	BMSC-EV	To investigate BMSC-EV therapeutic approaches in reducing AD burden	BMSCs-EV has neuroprotective properties by degrading Aβ.

Cui et al. (2019)	In-vivo	Mouse-APP/PS1	BMSC-exo	To investigate the effect of modified MSC-derived exosomes on cognitive behaviors of APP/PS1 mice.	BMSCs-exo as neuroprotective properties by degrading Aβ and regulating the inflammatory microglial expression
Reza-Zaldivar et al. (2019)	In-vivo	Mouse-C57BL/6	MSCs-exo (not specified)	To investigate MSC-exo therapeutic approaches in reducing AD burden	MSCs-exo increase neurogenesis by increasing neural precursor markers

4.5 Risk of bias assessment

For selection bias, three studies out of 11 mentioned randomization; however, the method of randomization was not elucidated, leading to a potentially low risk of bias, while eight studies recorded a potentially high risk of bias as they did not provide enough information on randomization. As for allocation concealment, a potentially high risk of bias is seen in allocation concealment as none of the studies mentioned enough information regarding it.

In performance bias, four studies showed a definitive low risk of identical experimental conditions, where there is direct proof that the vehicle was used in both control and test group. Four other studies revealed a potentially low risk of bias as there is indirect mentioning of the use of a vehicle, so identical conditions are assumed. However, the three remaining studies revealed a potentially high risk of bias as they did not elaborate on the vehicle used. As for blinding of researchers during studies, no record was found regarding it, leading to a potentially high risk of bias.

For attrition bias , all of the studies revealed a high bias of missing data outcome, as there is limited information regarding the loss of samples or animals throughout the studies.

In detection bias , the domains exposure characterization revealed a potentially high risk of bias in all studies as no record was found regarding it. All the studies were definitely low risk of bias in the following domains: outcome assessment and outcome reporting domains. In blinding of outcome assessors, all the studies had potentially a low risk of bias. Table 5 describes the results of the risk of bias assessment in all 11 studies.

Table 4-4 Results of risk of bias assessment for all studies

Risk of Bias Question		Ahmed et al (2016)	Lee et al (2018)	De Godoy et al (2017)	Wei et al (2020)	Ding et al (2018)	Nanako et al (2020)	Ma et al (2020)	Wang et al (2017)	Elia et al (2019)	Cui et al (2019)	Reza-Zaldivar et al (2019)
selection bias	Randomization	NR	NR	NR	NR	NR	NR	+	NR	NR	+	+
	Allocation concealment	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Performance bias	Identical experimental conditions	NR	++	+	NR	+	++	++	++	+	NR	+
	Blinding of researchers during study	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Attrition bias	Missing outcome data	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Detection bias	Exposure characterization	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Outcome assessment	++	++	++	++	++	++	++	++	++	++	++
	Blinding of outcome assessors	+	+	+	+	+	+	+	+	+	+	+
Reporting bias	Outcome reporting	++	++	++	++	++	++	++	++	++	++	++

4.6 Isolation & characterization methods for MSC-exo

4.6.1 Isolation methods

63.63% of total studies (7/11) used ultracentrifugation (Lee et al., 2018 ; De Godoy et al., 2017 ; Wei et al., 2020 ; Ma et al., 2020 ; Cui et al., 2019 ; Reza-Zaldivar et al., 2019), as for the isolation method of MSC-exo/EV; each of the remaining studies utilized magnetic capture-based Phosphatidylserine (PS) isolation method (Nanako et al.,2020), ExoQuick-TC (Ding et al., 2018), and polyethylene glycol (PEG) precipitation (Wang et al., 2017) (Figure 12A). In all of the studies included, the concentration of exosomes was referred to as protein concentration, concentration was usually referred as microgram per milliliter (ug/ml) or microliters (ul). Three studies (3/11, 27.27%) had an exosome concentration of 5ug/ml Studies (Ahmed et al., 2016 ; Ma et al., 2020 ; Reza-Zaldivar et al., 2019); concentrations varied between 5ug/ml – 200ug/ml and 4ul-100ul (Table 7). Only one study referred to exosome by actual number of exosome (De Godoy et al., 2017), two concentration were found**:** EV1,8×10^7 and EV2 2.4×10^8 .

4.6.2 Characterization method

4.6.2.1 TEM and NTA

The characterization methods of MSC-exo/EV include transmission electron microscopy (TEM) and Nanoparticle tracking analysis (NTA) (Figure 12B). Out of eleven studies, four studies (36.36%) used TEM as its morphology-based characterization method, while five (45.45%) used NTA to quantify exosome protein concentrations and measure its size distribution. Exosome size ranged between 30-200nm (Table 7). The biggest size of exosome found was 200nm, and it was found in

two studies (De godoy, et al., 2017; Wang et al., 2017). Smallest size found for exosomes was 30nm and it was found in six studies (Lee et al., 2018; De Godoy et al., 2017; Wei et al., 2020 ; Ding et al., 2018 ; Cui et al., 2019; Reza-Zaldivar et al., 2019).

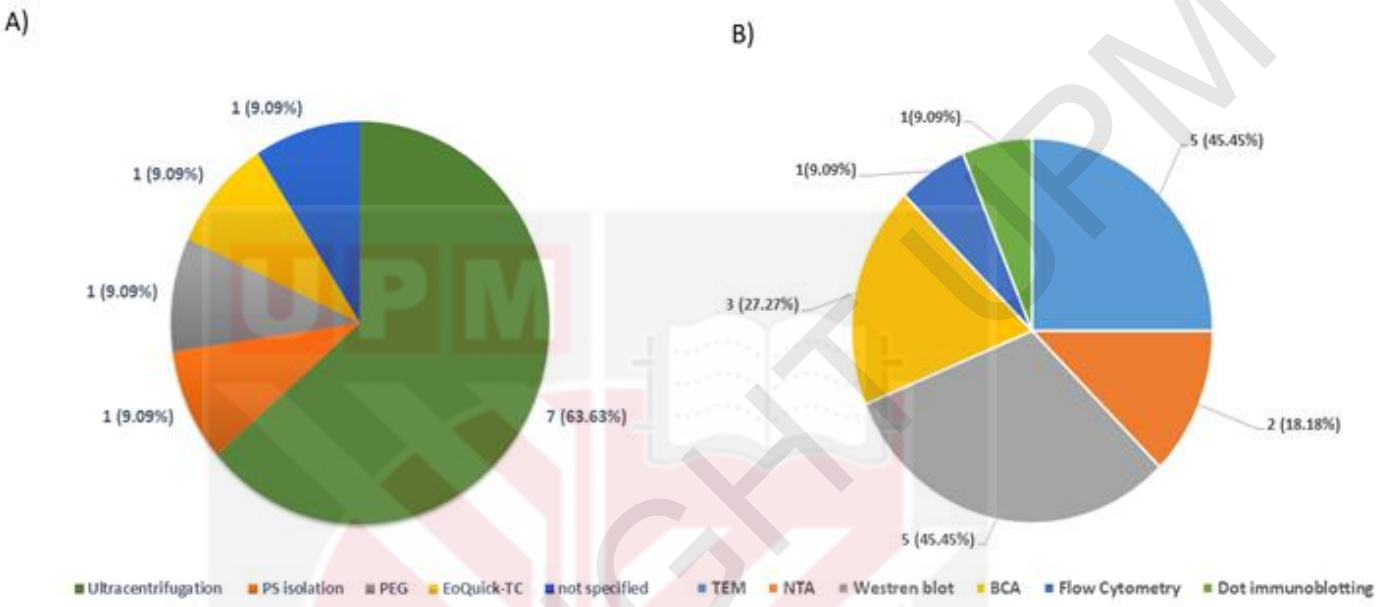

Figure 4-3 isolation and characterization methods of exosomes found in the studies

4.6.2.2 Surface marker expression

Commonly expressed exosomal surface markers are CD81, CD63, CD9, TSG101, Alix, and HSP70. The characterization method of MSC-exo/EV can be based on protein content using western blot, flow cytometry BCA assay, or dot immunoblotting. Five out of eleven (45.45% of total studies) used western blot as its protein-based characterization method, three studies (27.27% of all studies) used BCA assay. One study (9.09% of total studies) used flow cytometry, and another study (9.09% of total studies) used Dot immunoblotting. The marker CD81 was expressed in three studies. CD63 was expressed in five studies, CD9 was expressed in four

studies. The markers TSG101, Alix, and HSP70 were described in two studies. Detailed summary of the isolation method and characterization is described in Table 6.

Table 4-5 summary of study characteristics

Author and year	Source of exosomes/EV	Method of isolation and characterization	Size/protein concentration of exosomes	Markers of exosomes	No of samples/ animals
Ahmed et al. (2016)	DPSCs	**Isolation: - Characterizaitio n:** MAGPIX Cytokine Multiplex	**Size**:- **protein concentration**: 5 μg/mL	-	4 samples
Lee et al (2018)	ADSCs	**Isolation:** Ultracentrifugati on **Characterizaitio n:** Western blot,BCA assay	**Size**:30-100nm **protein concentration**: 200 μg/mL	HSP70, CD63, and CD9	3 samples
De Godoy et al (2017)	BMSCs	**Isolation:** Ultracentrifugati on **Characterizaitio n:** TEM, Dotimmunoblotti ng, Flow cytometry:	**Size**: 30-200nm **protein concentration**: Two concentration EV1,8 $\times 10^7$ EV2 2.4×10^8	CD81 and CD63	3 samples
Wei et al (2020)	HUCMS	**Isolation:** Ultracentrifugati on **Characterizaitio n:** TEM, NTA western blot,	**Size**: 30-120nm **protein concentration**: 2 µg /ml	CD63, CD81, tubulin	6 samples
Nanako et al. (2020)	BMSCs	**Isolation:** magnetic capture **Characterizaitio n: -**	**Size**:- **protein concentration**: -	-	1 sample
Ding et al (2018)	HUCSC	**Isolation:** ExoQuick-TC **Characterizaitio n:** western blot,TEM	**Size**: 30-150nm **protein concentration**: 30 µg/mL	CD9 and CD63	n= 12 (6 per group)
Ma et al (2020)	ADSCs	**Isolation:** ultracentrifugatio n **Characterizaitio n:** Western blot,TEM,NTA, Sucrose gradient	**Size**: 100nm **protein concentration:** -0.05 µg/ml, -0.1 µg/ml, -1 µg/ml -5 µg/ml	CD9, TSG101 and Alix	n=10 (5 per group)
Wang et al. (2017)	BMSCs	**Isolation:** PEG precipitation	**Size** 50-200nm	-	In-vitro: 2 groups (6 per group)

		Characterizaitio n: BCA	**protein concentration**: 100 µg/ml		In-vivo: 4 groups (7 per group)
Elia et al (2019)	BMSC	**Isolation:** ultracentrifugatio n **Characterizaitio n:** NTA, electro microscope QT-PCR, BCA assay, flow-cyto, and western blot	**Size** 100-150nm **protein concentration**: 4 µl	Alix, HSP70, TSG101, CD63 and CD9	Four groups (5-6 per group)
Cui et al (2019)	BMSC	**Isolation:** ultracentrifugatio n **Characterizaitio n:** NTA, immunofluoresce nce	**Size** 30-100nm **protein concentration**: 100 µl	-	Three groups (5 per group)
Reza-Zaldivar et al (2019)	MSCs	**Isolation:** Ultracentrifugati on **Characterizaitio n:** Westren blot, DC protein assasy	**Size** 30-100nm **protein concentration**: 5 µg/ µL	CD81	3 groups(n=12) 1 group (n=8)

4.7 Neuroprotection mode of actions using MSC-exo

Based on analysis of the 11 articles, various modes of action of MSC-exo/EVs in ameliorating AD burden were discovered (Figure 13). The mode of actions include: Immunomodulation, Reducing Apoptosis, Neurogenesis Degrading Aβ plaque, Delivery of miRNA and Ameliorating oxidative stress. Degrading Aβ plaque is the most common therapeutic mode of action among the studies (5/11, 45.45%), while delivery of miRNA and ameliorating oxidative stress were the least common (2/11, 18.18%).

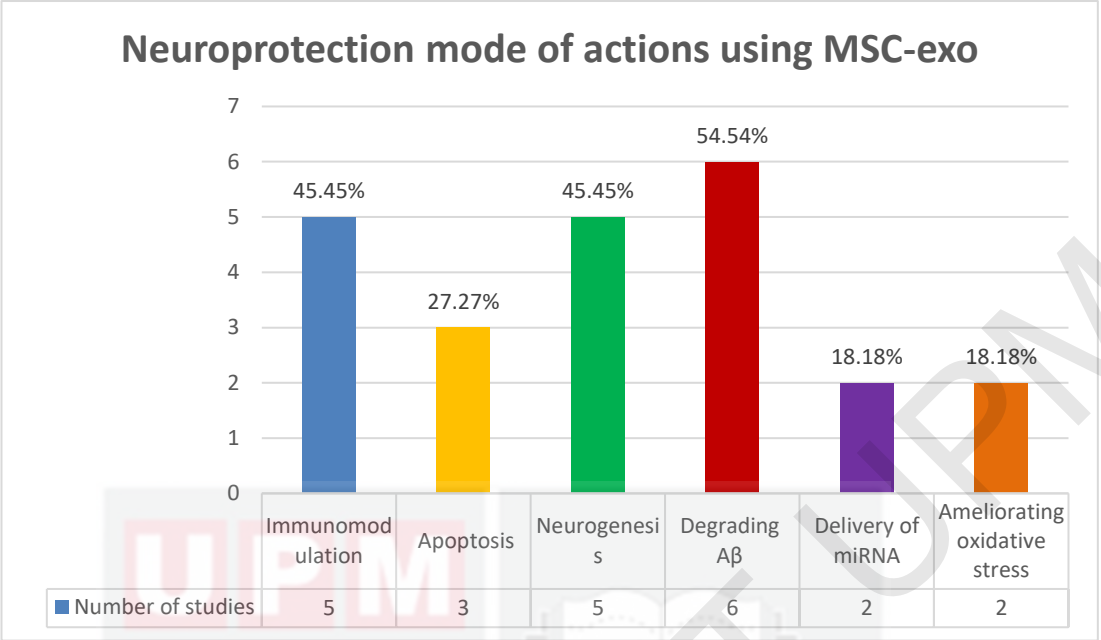

Figure 4-4 The various neuroprotective mode of action by MSC-Exo. Total number of studies included (n=11)

4.7.1 Alleviation of Neuroinflammatory response

Five studies out of eleven (45.45%) suggested that MSC-Exo ameliorated neuroinflammation. Two studies suggested a neuroprotective approach through reducing classical activation (M1), as Iba1, a marker of microglial activation, was reduced (Ma et al., 2020; Ding et al., 2018)Along with microglial inhibition, a study suggested an increase in alternative microglia (M2) activation as the ym-1 and Arg-1 genes increase in alternative microglia (Ding et al., 2018). Another finding indicated that MSC-exo delivers anti-inflammatory factors (Cui et al., 2019;), and decrease pro-inflammatory factors (Cui et al., 2019; Wei et al., 2020) . Two studies showed inhibition of astrocytes activation (Nanako et al., 2020 ; Cui et al., 2019).

4.7.2 Apoptosis and neurogenesis

Apoptosis and neurogenesis properties of MSC-exo were assessed. Three studies out of eleven (27.27%) revealed regulation of apoptotic factors (Ahmed et al.,

2016; Lee et al, 2018; Wei et al., 2020). In the AD model treated with MSC-exo, expression of anit-apoptotic genes such as Bcl-2 was upregulated, while pro-apoptotic genes such as Bax were downregulated (Ahmed et al., 2016). Five studies out of eleven (45.45%) indicated that MSC-exo increased neurogenesis by increasing neural precursor markers (Reza-Zaldivar et al., 2019), neutrile growth (Lee et al., 2018), increased synaptic density (De Godoy et al., 2017), and increased cell migration (Wei et al., 2020). One showed amelioration of dysmorphic neurites (Elia et al.,2020).

4.7.3 Degrading of Aβ

Six studies out of eleven (54.54%) revealed a reduction in Aβ plaque or Oligomers (Ahmed et al., 2016; Lee et al., 2018; Ding et al., 2018; Elia et al., 2020; Ma et al., 2020). few study suggested that the decline might be due to the act of degrading enzymes such as neprilysin (NEP) (Ahmed et al., 2016; Ding et al.,2018; Ma et al., 2020). Aβ levels in the hippocampus and cortex were assessed in in-vivo studies using thioflavin stain (Ding et al., 2018) and immunohistochemical staining (Elia et al., 2020; Ma et al.,2020).

4.7.4 delivery of miRNA

Two studies out of eleven (18.18%) revealed a neuroprotective property of miRNA against AD pathology through reducing neuroinflammation (Nanako et al., 2020) and apoptosis (Wei et al., 2020). In one study, miR-223 derived from HUC-exo showed an anti-apoptotic property by counteracting PTEN-PI3K/Akt pathway (Wei et al., 2020). Another miRNA derived from BMSC-exo, mi146a exhibited an anti-inflammatory property in astrocytes, as it reduced pro-inflammatory factors found in activated astrocytes (Nanako et al., 2020).

4.7.5 Ameliorating oxidative stress

Two studies out of eleven (18.18%) documented a relief of AD pathology by targeting oxidative stress (De godoy et al.,2017; Wang et al., 2017). One study used BMSC-Exo to target Nitric oxide-induced oxidative stress by reducing the expression of the Inducible Nitric Oxide Synthase (iNOS) enzyme (Wang et al., 2017). Another study used BMSC-EV to target hydrogen peroxide (H_2O_2) induced oxidative stress. This study revealed that BMSC-EV ameliorated oxidative stress by delivering catalase, an enzyme that counteracts reactive oxygen species derived from (H_2O_2)(De godoy et al.,2017).

CHAPTER 5

DISCUSSION

5.1 Sources of MSCs for AD research

The sources of MSCs derived exosomes found in this study were: bone marrow stem cells (BMSCs), adipose tissue stem cell (ADSCs), human umbilical cord stem cell (HUCSCs), and dental pulp stem cell (DPSCs). MSCs generally secrete paracrine factors residing in extracellular vesicles and secretomes which functions as immunomodulatory or anti-apoptotic factors (Phan et al.,2018; Xia et al., 2019). Based on our findings, most of the studies showed BMSCs as the main source of exosomes (45.45%); this might be due to BMSCs being the first MSCs to be discovered (Friedenstein et al., 1970) which leads to the abundance of the work on BMSCs. Methods of injecting MSC-Exo in AD models include intracerebroventricular injection (ICV), intravenous injection (IV), and intranasal injection. ICV accounted for the most method of injections among the studies (27.27%).

Advantages of each route of administration include directly transferring therapeutic agents into the central nervous system for ICV (CNS) (Glascock et al., 2011). The ability to titrate substance in intravenous injection (IV) (Shimizu, 2004). As for intranasal injection, advantages include crossing Blood Brain Barrier (BBB) and being a less invasive route (Upadhyay, 2011). Disadvantages of IV and ICV include discomfort and pain (Shimizu, 2004), disadvantage of intranasal route include fast elimination of substance due to mucosal clearance (Musumeci et al., 2019).

As for AD mice model age used in in-vivo research, studies used varying ages ranging from 8-7 weeks to 13 months. Variation in age might be due to animals being

studied at different stages of AD pathology. For instance, nine weeks old APP/PS1 begins to exhibit Aβ formation (Li & Wei, 2015) while around 15 months of age, AD behavioural characteristics begin to manifests (Olesen et al., 2016). Therefore, there is a need to establish an optimum age of animals to investigate. In in-vitro studies, most of the studies incubated MSC- exos ranging from 24hr-48hrs. But inadequate information was provided regarding setting this duration to test MSC- exos effects.

5.2 Characteristics of MSC- exosomes

Firstly, exosomes were isolated from MSCs. Among the research papers that have been reviewed, ultracentrifugation was the most common method for isolation (63.63%). Ultracentrifugation was introduced by Raposo et al. (1996), where exosomes were extracted from the B cell line. His protocol consists of continuous centrifuging at different centrifugal forces to obtain exosomes pellet. Ultracentrifugation is commonly used in exosomes studies as it focuses on the extraction of the smallest secretomes, exosomes (Szatanek et al.,2015). Only one study referred to exosomes among the research articles by the number of exosomes (de Godoy et al., 2017). Referring to exosomes using protein concentrations might be due to the difficulties in quantifying the actual number of exosomes. Other studies characterized exosomes by protein concentrations using Nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). TEM characterizes exosomes based on morphology, while NTA characterizes them based on concentration and size distribution. In surface marker expression, exosomal markers CD81, CD9, and CD63 were found among the majority the studies. In addition to tetraspanins function as a cell-surface marker of MSC-exo, studies showed that CD9 and CD81 had a role in α-secretase activation. CD9 and CD81 stimulated the

ADAM10 gene (Seipold & Saftig, 2016). ADAM10 catalyzes α-secretase activity leading to the cleavage of an sAPP-α fragment, subsequently reducing the accumulation of sAPP-β (amyloid-beta) fragments in the brain (Peron et al, 2018).

5.3 Therapeutic mode of actions of MSC-exosomes in AD

In the past two decades, there has been extensive work emphasizing the therapeutic mechanism of AD treatment. Work conducted on discovering tools for Aβ plaque reduction was highlighted as it was the primary causative of AD. Various researchers studied BACE1 inhibitors to prevent the accumulation of Aβ plaque; one study revealed that mice model deficient with BACE1 showed reduced memory defects (Ohno et al.,2014).

In our study, some researchers suggested the reduction of amyloid beta (Aβ) plaque through the act of degrading enzymes found in MSC-exo, such as Neprisilyn (NEP) and Insulin degrading enzyme (IDE). In a study conducted by Ahmed et al (2016), Dental pulp stem cell (DPSCs) secretomes were revealed to reduce Aβ42 oligomer by the act of enzyme NEP. Another study using human umblical cord stem cell (HUCSCs) derived-exosomes reduced Aβ plaque by the act of degrading enymes NEP and IDE (Ding et al, 2018). Similar findings were found in a study conducted by Katsuda et al. (2013). In a study conducted by Carpentier et al. (2002), an inverse relationship between NEP and Aβ was revealed, where NEP enzyme reduction resulted in the accumulation of Aβ in the cerebral vasculature. However, in other studies, measuring NEP levels was not an effective method as in some cases, the enzyme levels in both control and test groups did not differ as much (Hellström-Lindahl et al., 2008; Mattson, 2004). As for IDE enzymes, intensive research revealed

a correlation between AD and IDE, where the levels of Aβ plaque were reduced upon the introduction of IDE (Zhao et al., 2007; Pérez et al.,2000).

Another heavily investigated therapeutic mode of action was the neuroinflammatory response. Various players trigger a neuroinflammatory response in the CNS, including glial cells such as microglia and astrocytes or cellular injury. In our study, almost half of the papers revealed that MSC-Exo exhibited a mediation effect of neuroinflammation through various mechanism such as reducing oxidative stress (De godoy et al.,2017; Wang et al., 2017), inhibiting activated microglial (Ding et al., 2018; Ma et al., 2020) ,and astroglial activation (Nanako et al., 2020; Cui et al., 2019. Similar findings were revealed in a study conducted by Ni et al (2019) using traumatic brain injury. MSC-Exo treated the neuroinflammation by reducing activated microglial activity (M1) and the increase of alternative microglial activity (M2). It is thought that the composition of the exosome itself plays a huge role in their therapeutic property. For instance, MSC-derived exosomes tend to have anti-inflammatory cytokines and cargo protein components that make them a suitable candidate for targeting neuroinflammation (Reza-Zaldivar et al.,2018). Another finding found in our research is that MSC-exo reduced oxidative stress in AD models through catalase enzyme activity. A similar result was revealed in a study conducted by Bodart-Santos et al. (2019), where AβO induced oxidative stress was restored by hMSC-EV.

On top of its immunomodulatory effects, 27.27% of our findings revealed that MSC-exo exhibits neuroprotective properties by downregulating pro-apoptotic genes and upregulating anti-apoptotic genes. A study on spinal cord injury revealed similar results where the levels of the antiapoptotic gene Bcl-2 were upregulated upon the administration of MSC-Exo (Li et al., 2019). Low mi223 serum was associated with

AD expression (Jia & Liu,2016). In 18.18% of our findings, delivery of miRNA was used as a mechanism to counteract neuroinflammation and apoptosis. Mi223 balances PTEN-PI3K/Akt pathway by downregulation of PTEN and upregulation of p-Akt. A finding by Wen et al. (2020) revealed a similar outcome using BM-MSCs-Exo derived mi144 in cardiomyocyte apoptosis. The study conducted by Nanako et al. (2020) is the first study to reveal mi146a therapeutic effects of AD; as the previous finding showed either no significant difference (Maffioletti et al., 2020), upregulation of mi146a in AD patients (Lukiw et al., 2008), or downregulation of mi146a expression in AD patients (Schonrock et al., 2010).

Several outcome assessment methods were used for both in-vitro and in-vivo studies to assess MSC-exosome's effect on AD. Flow cytometry was one method used frequently to test the presence of the apoptotic population in the culture medium (Kummrow et al., 2013). One of the methods used in in-vivo studies was behavioural test; Morris water maze (MWM) is considered the most common method for behavioural testing to assess hippocampus function and spatial learning (Bryan et al., 2011).

5.4 Risk of bias assessment tool

In this review, the risk of bias was assessed using the OHAT tool. five risks of bias domain were addressed in this study. Overall, papers provided various levels of bias. Firstly, in selection bias, two questions addressed: random sequence generation and allocation concealment. These questions are addressed to confirm the intervention is not administered based on favourable characteristics. In our study, only three papers mentioned randomization with a potentially low risk of bias, while none of the papers

mentioned adequate information on allocation concealment. A potentially high risk of bias might lead to internal validity threats in the selection bias domain.

As for performance bias, the following two questions are addressed: identical experimental conditions and blinding of researchers during study. Identical experimental conditions are usually similar in animal studies; therefore, using a vehicle in the experimental and control group is usually the determinant of bias in this domain. (e.g., PBS was used in both experimental and control groups). In blinding of researchers during studies, researchers are blinded to avoid choosing animal based on desired charactersitics.

In attrition bias, missing data outcomes are addressed by providng information such as loss of sample or animals is addressed, in some studies, loss of animals or samples might influence the validity of the results.

In detection bias:, information regarding, exposure characterization, outcome assessment and blinding of outcome assessors is addressed. In exposure characterization, the purity of the test substance is measured using valid and reliable methods and given a specific score that indicates its purity. None of the papers assessed provided sufficient information regarding exposure characterization. The limited information might be due to the utilisation of MSC-Exo being a relatively new approach, and no exposure characterization has been established yet. The next question addressed is outcome assessment; this question assesses if well-established methods were used to determine the intervention. In our review, definitely low risk of bias was revealed in all studies. A well-established method was utilized to assess MSC-exo, such as NTA, TEM (Glushakova et al., 2018; Gurunathan et al., 2019). Another question evaluated in this domain is the blinding of outcome assessors; all our studies revealed a probably low risk of bias. This result is assumed as lack of blinding might

not result in bias (e.g., measurement in the studies are not subjective), or there is a need for outcome assessors to judge the outcome through comparing the two groups (e.g., comparing histological tissue of hippocampus and cortex of group treated with MSC-exo and untreated group).

Lastly, reporting bias assesses possible deviation of the study protocol by comparing paper outcome reporting with the methodology used. A definitive low risk of bias was determined in all papers as direct evidence of outcome being reported in abstract, methodology, and study protocol was provided.

CHAPTER 6

CONCLUSION, LIMITATION AND FUTURE RECOMMENDATIONS

6.1 Conclusion

The main aim of this work is to provide evidence that mesenchymal stem cells derived exosomes (MSCs-exo) in the treatment of Alzheimer disease (AD) through conducting a systematic review. The study searched through databases for relevant research on MSC-exo and AD using a detailed protocol. Potential sources and characteristics of MSCs exosomes were provided in addition to neuroprotective therapeutic actions on AD. Neuroprotective mode actions were immunomodulation, degrading amyloid plaque, reducing apoptosis, and delivery of miRNA.The findings of this study offer insights into the acellular therapy approach, specifically for AD treatment, by providing a novel method that deals with the pathological characteristics of AD.

6.2 Limitations

Our work was able to uncover abundant finding regarding MSCs-exo effects on AD, however certain limitations and obstacles were witnessed. One of the limitations is the search engines. In our study, only three search engines were targeted, which are PubMed, Scopus, and Medline. Covering more search engines might have resulted in other essential findings. Another limitation includes the time frame of our search. Search has been limited to Dec 2020, being the last date of publication. Several months have passed since then, and new findings with enhanced outcomes might have been published. Besides search engines, there is a difficulty in identifying quantities

of exosomes since the dose is based on protein concentrations. Other limitations were detected in bias assessments which are possible high risk of biases in randomization, allocation concealment, blinding, and exposure characterization method to assess the purity of MSC-Exo. Considerations are needed in terms of determining the appropriate animal age of AD animal models, duration of MSCs-Exo incubation in-vitro and defining factors influencing the efficacy levels of MSC-Exo, such as sex or age of the donor, is needed prior to AD research.

6.3 Future recommendations

Upon the completion of this work, we do see the likelihood of MSC-Exo research implementation in the near future. While appreciating the outcome of this work, we do see room for enhancement and improvement in the following aspects. Firstly, future research should focus on providing insight into factors influencing MSC-Exo efficacy before isolation. In addition to methods to quantify exosome and perhaps, lyophilization is used to preserve exosome after extractions. Another recommendation would be standardizing the protocol used for the MSC-Exo effect for AD research. Where the age of animals assessed in in-vivo studies, incubation period for in-vitro studies, isolation method, the dosage of MSC-Exo, and route of administration would be universal. And lastly, approaches to mitigate the biases must be implemented in preclinical studies. This can be done by randomizing the population tested using various randomization methods (e.g., sequence generation), making sure blinding is not broken when conducting the study, and providing an elaborated purity test of intervention for exposure characterization.

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APPENDICES