Investigating the biological effects of (nano)particles in Alzheimer's disease pathologies

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

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School of Life Sciences
Faculty of Science
University of Technology Sydney

Certificate of Original Authorship

I, Charlotte Fleming declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Science, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Statements

In accordance with the University of Technology Sydney thesis committee 'Graduate Research Candidature Management, Thesis Preparation and Submission Procedures (Version 1.10, 2021)', this PhD thesis is presented by compilation. It is comprised of two original Systematic and Bibliometric studies and two original Research studies submitted in peer reviewed journals of which I am the first author. I hereby declare that I have contributed significantly to these studies.

For the Systematic and Bibliometric studies, I procured all the data, performed systematic analysis of the papers identified and drafted the first copy of the two review papers.

For the Research studies, I carried out all experimental procedures, data analysis and drafted the first copy of the two research papers.

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Charlotte Fleming

22nd September 2022

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Publications

The following publications have arisen directly from work contained within this thesis.

Systematic and Bibliometric Analysis of Magnetite Nanoparticles and Their Applications in (Biomedical) Research.

Authors: Fleming, C.; Golzan, M.; Gunawan, C.; McGrath, K.

Accepted to Global Challenges. Manuscript no. gch2.202200009.

The Biological Roles of Air Pollutant Particulate Matters in the Early Onset Pathologies of Alzheimer disease.

Authors: Fleming, C.; Golzan, M.; Amal, R.; Wong, R.J.; Torpy, F.; Irga, P.; Gunawan, C.; McGrath, K.

Intend to submit to *ACS Nano*

Insights from a bibliometrics-based analysis of publishing and research trends on cerium oxide during 1990 – 2020

Authors: Fleming, C.; Wong, J.; Golzan, M.; Gunawan, C.; McGrath, K.

Intend to submit to *Dovepress*

Effects of cerium oxide nanoparticles in a mouse model of Alzheimer's disease exposed to magnetite pollution particles

Authors: Fleming, C.; Golzan, M.; Amal, R.; Esmailpour, A.; Gunawan, C.; McGrath, K.

Intend to submit to *ACS Nano*

Conference proceedings

New Horizons 2019

Charlotte Fleming, Peter Irga, Fraser Torpy, Cindy Gunawan, Mojtaba Golzan, Kristine McGrath. Investigating the Effects of Air Pollutant Nanoparticles in Alzheimer's Disease. 2019, Sydney, Australia.

FoSTER 2019

Charlotte Fleming, Peter Irga, Fraser Torpy, Cindy Gunawan, Mojtaba Golzan, Kristine McGrath. Investigating the Effects of Air Pollutant Nanoparticles on the Onset or Progression of Alzheimer's Disease. 2019; Sydney; Australia.

International Brain Research Organization 2019

Charlotte Fleming, Peter Irga, Fraser Torpy, Cindy Gunawan, Mojtaba Golzan, Kristine McGrath. Effects of Air Pollutant Particles in the Pathogenesis of Alzheimer's Disease. 2019, Daegu, Republic of Korea.

Graduate Research Symposium, 2021

Charlotte Fleming, Peter Irga, Fraser Torpy, Cindy Gunawan, Mojtaba Golzan, Kristine McGrath. Effects of Air Pollutant Particles in the Pathogenesis of Alzheimer's Disease. 2021, Malaysia.

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Table of Contents

List of Figures

List of Tables

Abbreviations

%	Percentage
µg	Micro gram
µl	Micro litre
µm	Micrometre
Aβ	Amyloid-Beta
ACEC	Animal Care & Ethics Committee
AD	Alzheimer's disease
AKI	Acute kidney injury
ATCC	Mouse embryonic stem cells
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APP	Amyloid precursor protein
APP/PS1	Human amyloid precursor protein (Mo/HuA695swe)/Presenilin 1 transgenic mouse model
BACE1	Beta-Secretase 1
BALB-C	Bagg Albino mouse model
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BV2	Murine microglial cells
β-actin	Beta-actin
β-secretase	Beta-secretase
Ca	Cornu ammonis
CAT	Catalase
cDNA	complementary deoxyribonucleic acid
CeO_2	Cerium oxide

Ce^{3+}	Cerium (oxidised)
Ce^{4+}	Cerium (reduced)
CO	Carbon monoxide
CNS	Central nervous system
CREB	cAMP-response element binding protein
C57BL/6	C57 black 6 mouse model
DE	Diesel
DCF	2'7'-Dichlorofluorescein
DG	Dentate gyrus
DMEM	Dublbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate polystyrene xylene
EDS	Ehlers-Danlos syndrome
EDTA	Ethylenediamine tetraacetic acid
EPM	Elevated plus maze
FBS	Foetal bovine serum
FCR	Field citation ratio
Fe_2O_3	Iron oxide
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
Fe_3O_4	Magnetite
GAPDH	Glyceraldyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
H_2O_2	Hydrogen peroxide
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HUVECs	Human umbilical vascular endothelial cells

HRP	Horseradish peroxidase
Iba-1	Ionized calcium binding adaptor molecule 1
ICAM-1	Intercellular adhesion molecule 1
IgG	Immunoglobulin G
IκB	Inhibitor of kappa B
IKKB	IκB kinase
IL-	Interleukin
iNOS	Inducible nitric oxide synthase
IRON	Iron and iron oxide air pollutant particles
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
MAG	Magnetite nanoparticles
MAPK	Mitogen-activated protein kinase
MCP-1	Macrophage chemoattractant protein-1
MDPI	Molecular diversity preservation international
MNPs	Magnetite nanoparticles
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MS	Multiple Sclerosis
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAC	N-acetylcysteine
NFκB	Nuclear factor-κB
NFTs	Neurofibrillary tangles
NIRF	Near infrared fluorescence
NMDA	N-methyl-D-aspartic acid
NO	Nitric oxide

NOR	Novel object recognition
NPs	Nanoparticles
OH^-	Hydroxide
OS	Oxidative stress
PD	Parkinson's disease
PM	Particulate matter
pMAPK	Phosphorylated MAPK
pmol	Picomole
pNFκB	Phosphorylated NFκB
pJNK	Phosphorylated JNK
pTau	Phosphorylated tau protein
qPCR	quantitative polymerase chain reaction
RIPA	Radioimmunoprecipitation assay buffer
ROI	Region of interest
ROS	Reactive oxygen species
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
SEM	Standard error mean
SOD	Superoxide dismutase
SPION	Superparamagnetic iron oxide nanoparticles
TBST	Tris buffered saline with tween
TEM	Transmission electron microscopy
TGX	Tris-glycine extended
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TST	Tail suspension test

TTau	Total tau protein
UV	Ultraviolet
WGS	Water-gas shift
WoS	Web of Science
W/T	Wild type
XRD	X-ray diffractometer
XPS	X-ray photoemission spectroscopy

Abstract

Alzheimer's disease (AD) is the most common form of dementia, with sporadic AD accounting for over 95% of cases and thought to be influenced by lifestyle and environmental factors. Magnetite pollutant particles have been found in abundance in brains of people with AD in densely populated cities. This observation highlighted the need for increased understanding of the potential impact on human health. Therefore, chapter 3 commenced an extensive systematic and bibliometric analytical review of the characteristics and applications of magnetite from 1990-2020, identifying the formation and broad applications in environmental, industrial, and biomedical fields, also highlighting the cytotoxic effects from overuse as a biomedicine and its potential implication in neurodegeneration and AD as an air pollutant. Subsequently, chapter 4 explored the biological effects that air pollutants (iron, diesel and magnetite) have on AD pathologies. This study showed air pollutants, particularly magnetite, increased anxiety, stress, and cognitive impairment, and increased neuronal cell loss in the hippocampus of the double transgenic APP/PS1 and W/T mice. Air pollutants also increased amyloid plaques and inflammation, in both the *in vivo* and *in vitro models*, neuroblastoma SH-SY5Y cells, with oxidative stress found to be induced via NFκB pathway, suggesting a global inflammatory response that occurs through activated microglial and astrocytes.

The current therapies for AD, while effective in managing symptoms do not delay or reverse disease progression. Oxidative stress is a central process in AD pathogenesis therefore the antioxidant, cerium oxide has emerged as a potential therapy. Cerium oxide has been used as a biomedicine for cancer therapy and ischemic stroke, however not for AD. This inspired the systematic and bibliometric review on cerium oxide from 1990-2020 (chapter 5), bringing to light the catalytic and redox properties used for innumerable environmental/industrial and biomedical applications. The advanced nanotechnology engineering was a focus in increasing nanoparticle efficiency for a wide range of applications, including AD. Consequently, because air pollutant magnetite induces AD pathologies, chapter 6 explored if cerium oxide nanoparticles could delay or reverse this. Cerium oxide nanoparticles decreased cognitive impairment, neuronal death, amyloid-beta species formation and inflammation in the APP/PS1 and W/T mice, and decreased inflammation and oxidative stress in SH-SH5Y and microglial BV-2 cells.

In summary, air pollutants induce neurological changes associated with AD, and after exposure to cerium oxide nanoparticles these changes are delayed or reversed. Overall, this study concludes that cerium oxide nanoparticles are promising potential therapeutics for air pollutant induced AD pathologies.