

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

Charlotte Fleming

September 2022

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.

Production Note:

Signature Signature removed prior to publication.

Date 22/09/2022

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.

Production Note:
Signature removed prior to publication.

Charlotte Fleming

22nd September 2022

Acknowledgments

First and foremost, I would like to acknowledge my principal supervisor, Dr Kristine McGrath, and co-supervisors Dr Cindy Gunawan and Dr Mojtaba Golzan for their continuous support and encouragement throughout my PhD. I am so thankful to have been their student and without their constant advice and expertise, I would not have produced the quality of work I have for this dissertation. Particularly Dr Kristine McGrath, who I have now been working with for the past 6 years. I truly admire you, and I would not have started or finished this without you. Thank you so much.

I want to express my sincere appreciation to Lal Overlunde and particularly Fiona Ryan, for taking care of my animals and their continuous encouragement and care while working in the Ernst Facility both for my experiments but also personally, you are both the best. I would like to thank Dr Catherine Gorrie for answering all my questions regardless of how silly they are and to all the help Associate Professor Louise Cole who always very patient in was assisting me with the Clarity clearing method. I would like to extend my gratitude to our collaborators – Professor Fraser Torpy and Dr Peter Irga, without which my project would not have been possible.

This study would not have been completed without the vast support from many research students and support staffs. I would like to thank the members of The Inflammation Team, Claire Rennie, Michael Chhor and Gihani Manadora for always being available to go for a coffee break, to discuss the most recent failed experiment, as well as their high-quality banter in the lab. In addition, I would like to thank my research friends Gerard Li, Daniel Turkewitz, Tara Nyugen and Alison Ricafrente for their support throughout my entire PhD. Thank you for your constant banter during our lunch breaks. I would like to also thank my housemates who have lived with me throughout my candidature who have always been there to hear me rant and complain and just for being there.

Finally, I would like to thank my family and friends. Lastly, and most importantly thank you Mum, Dad and Annabel for your unconditional love and support always. Words cannot describe how much I appreciate the hard work and dedication you have given to me; I would not have been able to do this without you.

Thank you everyone!

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.

Funding and Scholarships

This research was funded by the:

Australian Postgraduate Awards (APA) funded by the Australian Government, Department of Education and Training Scholarships

Table of Contents

| | |
|--|-----------|
| CERTIFICATE OF ORIGINAL AUTHORSHIP | I |
| STATEMENTS..... | II |
| ACKNOWLEDGMENTS..... | III |
| PUBLICATIONS | IV |
| <i>Conference proceedings</i> | v |
| <i>Funding and Scholarships</i> | vi |
| LIST OF FIGURES | XI |
| LIST OF TABLES | XIII |
| ABBREVIATIONS..... | XV |
| ABSTRACT..... | XX |
| CHAPTER 1: INTRODUCTION..... | 1 |
| CHAPTER SUMMARY | 1 |
| 1.1 ALZHEIMER’S DISEASE | 2 |
| 1.2 ALZHEIMER’S DISEASE PATHOGENESIS..... | 2 |
| 1.3 AIR POLLUTANT PARTICULATE MATTER AND NEURODEGENERATION | 6 |
| <i>1.3.1 Diesel exhaust air pollutant PM</i> | 8 |
| <i>1.3.2 Iron air pollutant PM</i> | 9 |
| <i>1.3.3 Magnetite air pollutant PM</i> | 9 |
| 1.4 CURRENT TREATMENT OPTIONS FOR ALZHEIMER’S DISEASE..... | 10 |
| 1.5 CERIUM OXIDE NANOPARTICLES AS A THERAPY FOR ALZHEIMER’S DISEASE..... | 11 |
| 1.6 SIGNIFICANCE | 13 |
| 1.7 AIMS AND HYPOTHESIS | 13 |
| CHAPTER 2: MATERIALS AND METHODS | 15 |
| CHAPTER SUMMARY | 15 |
| 2.1 SYNTHESIS OF PARTICLES..... | 16 |

| | |
|---|----|
| 2.2 TRANSMISSION ELECTRON MICROSCOPY (TEM) | 17 |
| 2.3 DOUBLE TRANSGENIC APP/PS1 <i>IN VIVO</i> MODELS..... | 17 |
| 2.4 PREPARATION AND ADMINISTRATION OF PARTICLES AND NANOPARTICLES FOR THE <i>IN VIVO</i> MODELS | 18 |
| 2.5 BEHAVIOURAL TESTING | 22 |
| 2.6 NEAR-INFRARED (NIRF) <i>IN VIVO</i> BRAIN IMAGING | 26 |
| 2.7 TISSUE HARVEST AND BLOOD COLLECTION | 27 |
| 2.8 BIOCHEMICAL ANALYSIS OF PLASMA | 27 |
| 2.9 TISSUE FIXATION, PROCESSING, EMBEDDING, AND CUTTING..... | 28 |
| 2.10 HISTOLOGICAL ANALYSIS OF THE HIPPOCAMPUS..... | 28 |
| 2.11 CELL CULTURE <i>IN VITRO</i> MODELS | 29 |
| 2.12 BIOCHEMICAL ASSAYS | 31 |
| 2.13 QUANTIFICATION OF GENE EXPRESSION BY RT-QPCR..... | 32 |
| 2.14 PROTEIN EXTRACTION | 36 |
| 2.15 AB42 ELISA..... | 36 |
| 2.16 WESTERN BLOT ANALYSIS..... | 37 |
| 2.17 STATISTICAL ANALYSIS | 38 |

CHAPTER 3: SYSTEMATIC AND BIBLIOMETRIC ANALYSIS OF MAGNETITE NANOPARTICLES AND THEIR APPLICATIONS IN (BIOMEDICAL) RESEARCH

| | |
|-------------------------------|-----------|
| | 40 |
| SUBMITTED AS: | 40 |
| CHAPTER SUMMARY | 40 |
| AUTHORS' CONTRIBUTIONS: | 41 |
| 3.1 ABSTRACT:..... | 42 |
| 3.2 INTRODUCTION..... | 43 |
| 3.3 RESULTS..... | 44 |
| 3.3 DISCUSSION..... | 62 |

| | |
|--|------------|
| 3.4 CONCLUSION | 68 |
| 3.5. METHODS..... | 69 |
| 3.6 SUPPORTING INFORMATION..... | 71 |
| CHAPTER 4: THE BIOLOGICAL ROLES OF AIR POLLUTANT PARTICULATE MATTERS IN THE EARLY ONSET PATHOLOGIES OF ALZHEIMER’S DISEASE | 73 |
| INTEND TO SUBMIT AS: | 73 |
| CHAPTER SUMMARY | 73 |
| AUTHORS’ CONTRIBUTIONS: | 74 |
| 4.1 ABSTRACT..... | 76 |
| 4.2 INTRODUCTION..... | 77 |
| 4.3 METHODS..... | 79 |
| 4.4 RESULTS AND DISCUSSION..... | 86 |
| 4.5 CONCLUSION..... | 107 |
| 4.6 SUPPORTING INFORMATION..... | 108 |
| CHAPTER 5: INSIGHTS FROM A BIBLIOMETRICS-BASED ANALYSIS OF PUBLISHING AND RESEARCH TRENDS ON CERIUM OXIDE DURING 1990-2020 | 114 |
| INTEND TO SUBMIT AS: | 114 |
| CHAPTER SUMMARY | 114 |
| AUTHORS’ CONTRIBUTIONS: | 115 |
| 5.1 ABSTRACT:..... | 118 |
| 5.2 INTRODUCTION..... | 119 |
| 5.3 MATERIAL AND METHODS | 119 |
| 5.4 RESULTS..... | 122 |
| 5.5 DISCUSSION..... | 140 |
| 5.6 CONCLUSION..... | 148 |

| | |
|--|------------|
| CHAPTER 6: EFFECTS OF CERIUM OXIDE NANOPARTICLES IN A MOUSE MODEL OF ALZHEIMER’S DISEASE EXPOSED TO MAGNETITE POLLUTION PARTICLES | 149 |
| INTEND TO SUBMIT AS: | 149 |
| <i>CHAPTER SUMMARY:</i> | 149 |
| AUTHORS’ CONTRIBUTIONS: | 150 |
| 6.1 ABSTRACT..... | 152 |
| 6.2 INTRODUCTION..... | 153 |
| 6.3 METHODS..... | 157 |
| 6.4 RESULTS AND DISCUSSION..... | 164 |
| 6.5 CONCLUSION..... | 190 |
| 6.6 SUPPLEMENTARY INFORMATION..... | 191 |
| CHAPTER 7: CONCLUSIONS AND FUTURE PERSPECTIVES..... | 198 |
| CHAPTER SUMMARY | 198 |
| 7.1 CONCLUSIONS | 199 |
| 7.2 FUTURE PERSPECTIVES..... | 204 |
| REFERENCES..... | 207 |
| REFERENCES | 208 |
| APPENDICES..... | 247 |
| APPENDIX 1: WESTERN BLOT IMAGES FOR <i>IN VIVO</i> STUDY 1 | 248 |
| APPENDIX 2: WESTERN BLOT IMAGES FOR <i>IN VITRO</i> STUDY 1 | 252 |
| APPENDIX 3: WESTERN BLOT IMAGES FOR <i>IN VIVO</i> STUDY 2 | 256 |
| APPENDIX 4: WESTERN BLOT IMAGES FOR <i>IN VITRO</i> STUDY 2 | 260 |
| APPENDIX 5: SYSTEMATIC AND BIBLIOMETRIC ANALYSIS OF MAGNETITE NANOPARTICLES AND THEIR APPLICATION IN (BIOMEDICAL) RESEARCH..... | 264 |

List of Figures

| | |
|------------------|-----|
| Figure 1.1..... | 8 |
| Figure 2.1..... | 19 |
| Figure 2.2..... | 21 |
| Figure 2.3..... | 23 |
| Figure 2.4..... | 25 |
| Figure 2.5..... | 26 |
| Figure 2.6..... | 34 |
| Figure 3.1..... | 46 |
| Figure 3.2..... | 50 |
| Figure 3.3..... | 52 |
| Figure 3.4..... | 53 |
| Figure 3.5..... | 62 |
| Figure 3.6..... | 63 |
| Figure 3.7..... | 71 |
| Figure S3.1..... | 72 |
| Figure 4.1..... | 87 |
| Figure 4.2..... | 92 |
| Figure 4.3..... | 95 |
| Figure 4.4..... | 99 |
| Figure 4.5..... | 104 |
| Figure S4.1..... | 108 |
| Figure S4.2..... | 111 |
| Figure S4.3..... | 112 |
| Figure 5.1..... | 121 |
| Figure 5.2..... | 123 |

| | |
|------------------|-----|
| Figure 5.3..... | 127 |
| Figure 5.4..... | 130 |
| Figure 5.5..... | 131 |
| Figure 5.6..... | 140 |
| Figure 5.7..... | 141 |
| Figure 5.8..... | 147 |
| Figure 6.1..... | 156 |
| Figure 6.2..... | 169 |
| Figure 6.3..... | 173 |
| Figure 6.4..... | 176 |
| Figure 6.5..... | 178 |
| Figure 6.6..... | 181 |
| Figure 6.7..... | 184 |
| Figure 6.8..... | 188 |
| Figure S6.1..... | 191 |
| Figure S6.2..... | 192 |
| Figure S6.3..... | 193 |
| Figure S6.4..... | 195 |
| Figure S6.5..... | 197 |

List of Tables

| | | |
|-------------|---|-----|
| Table 2.1. | Summary of treatment and genders grouped for animal study 1 | 19 |
| Table 2.2. | Summary of treatment and genders grouped for animal study 2..... | 22 |
| Table 2.3. | PCR Primer Sequences for the <i>in vivo</i> experiment..... | 36 |
| Table 2.4. | Antibodies used for Western Blot analysis..... | 38 |
| Table 3.1. | Summary of the number of papers identified in searches of different databases in the years 1990-2020. Databases Web of Science (WoS), PubMed® and Scopus were accessed on the 14 th December 2020 and covered the article, title, abstract and keywords.... | 45 |
| Table 3.2. | Top 19 topic models generated from PubMed dataset (938 publications) by SWIFT- Review software, using the search term “magnetite”. This search was refined to clinical trials, meta-analysis, review, and systematic review articles. The topics have been ordered by number of publications contributing to the topic model in descending order, with topic words and themes established. Accessed on 14 th December 2020. | 47 |
| Table 3.3. | Summary of word clusters identified using VOSviewer and the WoS dataset obtained using a search for the term “Magnetite”. The network analysis from 8, 529 publications from 1990-2020. The clusters are represented in a visualisation map (refer to Figure 3.2). Accessed on the 14 th of December 2020..... | 49 |
| Table S4.1. | Primary and secondary antibodies used in western blot analysis | 109 |
| Table S4.2. | PCR Primer Sequences for qPCR analysis | 110 |
| Table 5.1. | Summary of the number of papers identified in searchers of different databases (PubMed, WoS and Scopus) using the search terms “ <i>cerium oxide OR ceria OR nanoceria OR nano ceria</i> ” from the years 1990 - 2020. | 122 |
| Table 5.2. | Top 19 topic models generated from PubMed dataset (129 publications) by SWIFT-Review software using the search terms “ <i>cerium oxide OR ceria OR nanoceria OR nano ceria</i> ”. This search was refined to review, clinical trials, meta-analysis, and research articles. The topics have been ordered by number of publications contributing to the topic model in descending order, with topic words and themes established..... | 125 |

| | |
|--|-----|
| Table 5.3. Summary of the word clusters identified using VOSviewer and WoS dataset using the search term “ <i>cerium oxide OR ceria OR nanoceria OR nano ceria</i> ”. The network analysis from 7, 862 publications from 1990 - 2020. The clusters are also represented in a visualisation map (Figure 5.3). | 128 |
| Table S6.1. Antibodies used in Western Blot analysis | 194 |

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 | Dulbecco'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 ₂ O ₃ | Iron oxide |
| Fe ²⁺ | Ferrous ion |
| Fe ³⁺ | Ferric ion |
| Fe ₃ O ₄ | Magnetite |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| GFAP | Glial fibrillary acidic protein |
| H ₂ O 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 |
| I κ B | Inhibitor of kappa B |
| IKKB | I κ B 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 |
| NF κ B | Nuclear factor- κ B |
| 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 NF κ B 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.