Linköping Studies in Science and Technology
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Cerium Oxide Nanoparticles and Gadolinium Integration

Synthesis, Characterization and Biomedical Applications

Peter Eriksson

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Applied Physics

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Front cover: A cerium oxide nanoparticle with integrated gadolinium.

Back cover: Cross-section of a gadolinium-cerium oxide nanoparticle and its displayed theragnostic properties: left) cerium undergo redox-reactions to scavenge reactive oxygen species and right) gadolinium shorten the T_1 -relaxation time of nuclei spins in water molecules.

During the course of the research underlying this thesis, Peter Eriksson was enrolled in Forum Scientium, a multidisciplinary doctoral programme at Linköping University, Sweden.

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Cerium Oxide Nanoparticles and Gadolinium Integration; Synthesis, Characterization and Biomedical Applications

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Den här känslan av att vara ett fallande löv i vinden

Kjell Höglund

Abstract

A challenging task, in the area of magnetic resonance imaging is to develop contrast enhancers with built-in antioxidant properties. Oxidative stress is considered to be involved in the onset and progression of several serious conditions such as Alzheimer's and Parkinson's disease, and the possibility to use cerium-contained nanoparticles to modulate such inflammatory response has gained a lot of interest lately.

The rare earth element gadolinium is, due to its seven unpaired f-electrons and high symmetry of the electronic state, a powerful element for contrast enhancement in magnetic resonance imaging. Chelates based on gadolinium are the most commonly used contrast agents worldwide. When introducing external contrast agents there is always a risk that it may trigger inflammatory responses, why there is an urgent need for new, tailor-made contrast agents.

Small sized cerium oxide nanoparticles have electronic structures that allows co-existence of oxidation states 3+ and 4+ of cerium, which correlates to applicable redox reactions in biomedicine. Such cerium oxide nanoparticles have recently shown to exhibit antioxidant properties both *in vitro* and *in vivo*, via the mechanisms involving enzyme mimicking activity.

This PhD project is a comprehensive investigation of cerium oxide nanoparticles as scaffold materials for gadolinium integration. Gadolinium is well adopted into the crystal structure of cerium oxide, enabling the combination of diagnostic and therapeutic properties into a single nanoparticle. The main focus of this thesis project is to design cerium oxide nanoparticles with gadolinium integration. A stepwise approach was employed as follows: 1) synthesis with controlled integration of gadolinium, 2) material characterization by means of composition crystal structure, size, and size distribution and 3) surface modification for stabilization. The obtained nanoparticles exhibit remarkable antioxidant capability *in vitro* and *in vivo*. They deliver strongly enhanced contrast per gadolinium in magnetic resonance imaging, compared to commercially available contrast agents.

A soft shell of dextran is introduced to encapsulate the cerium oxide nanoparticles with integrated gadolinium, which protects and stabilizes the hard core and to increases their biocompatibility. The dextran-coating is clearly shown to reduce formation of a protein corona and it improves the dispersibility of the nanoparticles in cell media. Functionalization strategies are currently being studied to endow these nanoparticles with specific tags for targeting purposes. This will enable guidance of the nanoparticles to a specific tissue, for high local magnetic resonance contrast complemented with properties for on-site reduced inflammation.

In conclusion, our cerium oxide nanoparticles with integrated gadolinium, exhibit combined therapeutic and diagnostic, i.e. theragnostic capabilities. This type of nanomaterial is highly promising for applications in the field of biomedical imaging.

Populärvetenskaplig sammanfattning

En stor utmaning idag är att utveckla diagnostiska kontrastförstärkare med inbyggda antioxidativa egenskaper. Ceriumoxid nanopartiklar är ett mycket lovande antioxidativt material som också har visat sig fungera mycket bra som bärarmaterial för andra sällsynta jordartsmetaller. Gadolinium är en sällsynt jordartsmetall med utmärkta magnetiska egenskaper. Ett flertal gadolinium komplex används dagligen världen över som kontrastförstärkare inom magnetresonanstomografi. Gadolinium kan på ett mycket bra sätt integreras i ceriumoxids kristallstruktur, vilket resulterar i en kombination av både diagnostiska och terapeutiska egenskaper i en och samma nanopartikel.

Ceriumoxid nanopartiklar har en elektronstruktur som tillåter samexistens av cerium med två olika oxidationstillstånd (Ce^{3+} och Ce^{4+}). Balansen mellan dessa två oxidationstillstånd kan justeras i närvaro av fria radikaler. Det är kopplat till de nyligen påvisade effekterna av ceriums antioxidativa egenskaper. Gadolinium har, med sina 7 oparade 4f-elektroner och sin höga symmetri i elektronstrukturen, visat sig vara det mest effektiva grundämnet för att uppnå god kontrast inom magnetresonanstomografi.

Detta doktorandprojekt omfattar 1) syntes: 2) karaktärisering med avseende på storlek, form och storleksfördelning samt 3) yt-modifiering av ceriumoxid nanopartiklar med kontrollerad integrering av gadolinium. Dessa ceriumoxid nanopartiklar med integrerat gadolinium, påvisar anmärkningsvärda antioxidativa egenskaper både *in vitro* och *in vivo* och bidrar dessutom med kraftigt förstärkt kontrast per gadolinium inom magnetresonanstomografi i jämförelse med vad de kommersiellt använda kontrastmedlen bidrar med.

I ett andra steg i denna design av nanopartiklar för tillämpning inom magnetresonanstomografi, har vi kapslat in gadolinium-ceriumoxid nanopartiklarna med ett mjukt skal av dextran, som ska skydda och stabilisera kärnan och förbättra biokompatibiliteten. Vi har i detta arbete visat att dextraninkapslingen skyddar nanopartikelytan mot oönskad proteininbindning (proteinkorona). Dessutom förbättrar dextraninkapsling också partiklarnas förmåga att dispergera i cellmedium. Nya funktionaliseringsstrategier är nu i fokus för att utrusta nanopartiklarna med specifika funktionella grupper skräddarsydda för målsökning av en utvald vävnad. Nanopartiklarna kan då på plats, bidra med inflammationsdämpande egenskaper, samt leverera en hög, lokal magnetresonanskontrast.

Till sist bör det noteras att oxidativ stress anses vara involverad i igångsättning och fortsatt utveckling av inflammatoriska sjukdomstillstånd såsom när det gäller både Alzheimers - och Parkinsons sjukdom. Möjligheten att använda nanopartiklar med antioxidanta egenskaper för att modulera detta inflammationssvar har nyligen väckt stort intresse. Vår design av ceriumoxid nanopartiklar med integrerat gadolinium, syftar till att kombinera terapeutiska och diagnostiska egenskaper och detta har stor potential för tillämpning inom biomedicinsk utbildning.

Publication List:

Papers included in the thesis

Paper I

Peter Eriksson, Alexey A. Tal, Andreas Skallberg, Caroline Brommesson, Zhangjun Hu, Robert D. Boyd, Weine Olovsson, Neal Fairley, Igor A. Abrikosov, Xuanjun Zhang & Kajsa Uvdal, *Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement*, **Scientific Reports volume 8, Article number: 6999 (2018)**.

Author's contribution: PE synthesized the nanoparticles. PE was main responsible for planning, performing and evaluating following experimental work; DLS, Zeta potential, XRD, NEXAFS and the relaxivity. PE prepared the TEM samples and evaluated the TEM results together with RDB. PE wrote the main part of the paper.

Paper II

Peter Eriksson, Anh H.T. Truong, Caroline Brommesson, Ganesh R. Kokil, Robert D. Boyd, Zhangjun Hu, Tram T. Dang, Per Persson, & Kajsa Uvdal, *Cerium Oxide Nanoparticles with Entrapped Gadolinium for High T1 Relaxivity and ROS-scavenging Purposes*, **submitted to ACS Omega**.

Author's contribution: PE synthesized the nanoparticles. PE was responsible for planning, performing and evaluating following experimental work, DLS, Zeta potential, XRD, relaxivity, Optical spectroscopy and XPS. PE prepared samples for STEM-EELS and Raman spectroscopy. PE was responsible for evaluating the Raman spectroscopy results. PE wrote the main part of the paper.

Paper III

Maria Assenhöj, **Peter Eriksson**, Pierre Dönnès, Stefan A. Ljunggren, Kajsa Uvdal, Helén Karlsson & Karin Cederbrant, *Combining Efforts to Evaluate the Toxicity of Metal Oxide Nanoparticles in Human Monocytes and Plasma*, **in manuscript**.

Author's contribution: PE synthesized nanoparticles for this paper. PE was responsible for planning, performing and evaluating the DLS work. PE contributed to the manuscript.

Paper IV

Peter Eriksson, Maria Assenhøj, Caroline Brommesson, Kalle Bunnfors, Reza Nosratabadi, Stefan A. Ljunggren, Helén Karlsson & Kajsa Uvdal. *Gd-CeNPs functionalized with FITC-conjugated dextran for enhanced T1 relaxivity, reduced protein adsorption and ROS scavenging properties*, **in manuscript**.

Author's contribution: PE synthesized the nanoparticles. PE was responsible for planning, performing and evaluating the following experimental work; DLS, Zeta potential, TEM, FTIR-ATR, TGA and relaxivity. PE was involved in planning, performing and evaluating the antioxidant assay together with CB. PE wrote the main part of the paper.

Paper V

Peter Eriksson, Caroline Kardeby, Caroline Brommesson, Eva Lindström, Magnus Grenegård & Kajsa Uvdal. *A pilot study on Gadolinium-Integrated Cerium Oxide Nanoparticles in Whole Blood; Platelet Aggregation and Inhibition of ROS-Production*, **in manuscript**.

Author's contribution: PE synthesized the nanoparticles. PE was responsible for planning, performing and evaluating the following experimental work; DLS, Zeta potential, relaxivity, TEM of the nanoparticles, AFM and SEM-EDX. PE wrote the main part of the paper.

Paper VI

Kalle Bunnfors, Natalia Abrikossova, Joni Kilpijärvi, **Peter Eriksson**, Jari Juuti, Niina Halonen, Caroline Brommesson, Anita Lloyd Spets & Kajsa Uvdal, *Nanoparticle activated Neutrophils-on-a chip; A label-free capacitive sensor to measure cells at work*, **submitted to Sensors and Actuators B**.

Author's contribution: PE synthesized the nanoparticles. PE was responsible for the planning, performing and evaluating the TEM and SEM work. PE contributed to the manuscript.

Related papers, not included in thesis:

Zhangjun Hu, Guangqing Yang, Jiwen Hu, Hui Wang, **Peter Eriksson**, Ruilong Zhang, Zhongping Zhang & Kajsa Uvdal, *Real time visualizing the regulation of reactive oxygen species on Zn 2+ release in cellular lysosome by a specific fluorescent probe*, *Sensors & Actuators B*, **volume 264**, 2018.

Linnéa Selegård*, **Peter Eriksson***, Zhangjun Hu & Kajsa Uvdal, *Corrosion and ice adhesion protection of Alumina oxide surfaces using cerium oxide sealing*, **in manuscript**.

** These authors contributed equally to this work*

Conference contributions

Oral Presentations

Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement

Peter Eriksson, Zhangjun Hu, Caroline Brommesson, Xuanjun Zhang, Kajsa Uvdal
2nd Asia Pacific Nano Biotechnology Summit, 29-30 June 2017, Singapore, Singapore

Cerium oxide nanoparticles with antioxidant capabilities carrying gadolinium and europium

Peter Eriksson

Advanced Functional Materials, 23-24 August 2016, Kolmården, Sweden

Poster Presentations

Cerium oxide nanoparticles with antioxidant capabilities carrying gadolinium for MRI contrast enhancement

Peter Eriksson, Alexey Tal, Andreas Skallberg, Caroline Brommesson, Zhangjun Hu, Weine Olofsson, Robert Boyd, Neal Fairley, Igor Abrikosov, Xuanjun Zhang and Kajsa Uvdal

4th Macau Symposium on Biomedical Sciences, 21-2 June, Macau, Macau SAR China

Cerium oxide nanoparticles with antioxidant capabilities carrying gadolinium and europium

Peter Eriksson, Andreas Skallberg, Caroline Brommesson, Zhangjun Hu, Robert Boyd, Neal Fairley, Xuanjun Zhang and Kajsa Uvdal

Advanced Functional Materials, 23-24 August 2016, Kolmården, Sweden

Development of “all-in-one” theragnostic nanoprobe

Peter Eriksson, Andreas Skallberg, Caroline Brommesson, Xuanjun Zhang and Kajsa Uvdal

Advanced Functional Materials, 20-21 August 2014, Kolmården, Sweden

Workshops and Meetings

Infrared Chemical Imaging for the Future at MAX IV, 8-9 March 2017, Uppsala, Sweden

XPS Imaging, 17 March 2015, Teignmouth, United Kingdom

1st NanoESCA Training School, 8-10 October 2014, Grenoble, France

5th FOCUS PEEM Workshop, 13-15 November 2013, Hünstetten, Germany

Abbreviations

AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflectance
CT	Computed Tomography
DEG	Diethylene Glycol
DFT	Density Functional Theory
DLS	Dynamic Light Scattering
EDS	Energy Dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
FM	Fluorescence Microscopy
IR	Infrared Spectroscopy
FWHM	Full-Width at Half Maximum
MRI	Magnetic Resonance Imaging
NEXAFS	Near Edge X-ray Absorption Fine Structures Spectroscopy
PEG	Polyethylene Glycol
RE	Rare Earth
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
SOD	Superoxide Dismutase
TEG	Triethylene Glycol
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction

Preface

What do smartphones, speakers, and banknotes have in common? They all utilize the exceptional properties of the rare earth elements, which played an essential role in the technological developments that occurred during the last decade. In nature, these elements occur in groups of about four to five rare earths dispersed in the same ore and the properties of individual rare earth elements can easily be combined into a single material. The unique electronical, magnetic and optical properties of the rare earth elements are highly dependent on their size and modern nanotechnology provides the means to control and manipulate the size of nanoparticles and thereby introduce new and intriguing properties.

In this thesis, nanoparticles of the rare earth elements, cerium and gadolinium have been synthesized and utilized. Synthesized cerium oxide nanoparticles combined with gadolinium enables the combination of both antioxidant and MRI-contrast enhancing properties, hence this unique nanomaterial displays both therapeutic and diagnostic properties. In this work, I together with my colleagues have shown that cerium oxide nanoparticles with integrated gadolinium have great potential for several applications within biomedical imaging. This thesis is divided into five chapters. In chapter 1, the field of nanotechnology is introduced as well as the properties of rare earth elements, focusing on cerium and gadolinium. Further, the synthesis procedures and the methods utilized within this work, are described in Chapter 2 and the most significant results for the synthesized nanoparticles are presented in Chapter 3. A short summary of the individual papers is found in the Chapter 4. Finally, future perspectives of the gadolinium-integrated cerium oxide nanoparticles are presented in Chapter 5.

The main work of this thesis has been conducted within the group of Molecular Surface Physics and Nanoscience, The Department of Physics, Chemistry and Biology (IFM), Linköping University (LiU), Sweden. There are numerous people that have helped me both at work and in life during my years at IFM, and I would like to express my sincere gratitude and appreciation to some particular people.

First and foremost, I want to thank my main supervisor Kajsa Uvdal, for motivating me to push my research further. I will for sure remember our nice fika-discussions about finding ‘flow’ in work and life. Secondly, I want to show my appreciation to all my co-supervisors. Caroline Brommesson, for all the support with the biological assays in this thesis and for that you made me realize where I were when LiU was invaded by Tintin, Captain Haddock, stormtroopers and the other participants at NärCon ☺. Xuanjun Zhang, for directing me to work with cerium oxide nanoparticles and for hosting me during my three months visit at the University of Macau. Zhangjun Hu, for all the quick feedback and good discussions you and I have had in our daily work.

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Maria and Helen at AMM, Alexey and Igor in the group of Theoretical Physics, Per Persson, Magnus Grenegård and Robert Boyd who all have been giving valuable input for the research content in this thesis. Alexei Preobrajenski, I want to thank for all the service during the conducted NEXAFS studies at the beamline D1011 at Maxlab II in Lund.

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My brothers and their families; Johan and Maria and Henrik, Anna, Marit and Sigge for all lovely times we have had and will have. I am really looking forward to the next year’s fishing trip, which I hope will also include trainings in some good-looking “OL-Blusar” ☺.

Finally, I want to thank my beloved parents, Marie and Pär. I don’t know how many car rides to different trainings and competitions you have done for me. I will always be grateful for that you have given me the opportunity to pursue my dreams.

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1. Introduction

Nanoscience is the study of the fundamental concepts of matter in the nanoscale dimension (1-100 nm, Figure 1.1). The Nanotechnology is the manipulation of matter at a molecular or atomic level to produce new unique properties that significantly differ from the bulk properties. The concepts were earliest discussed in 1959 by Richard Feynman (*There's Plenty of Room at the Bottom*), debating the importance of atomic engineering and the enormous potential of miniaturization. The field of nanotechnology made major breakthroughs in the 1980's due to the development of electron microscopy, invention of scanning probe microscopy and the availability of more powerful computer power, which allowed complementary research activities of atomic-scale visualization and characterization and modeling and simulation. Nanoscience has gathered scientists from physics, chemistry, biology and engineering to work on a cross-disciplinary research platform and the field is today well-established in both academia and society all over the world^{1,2}.

Figure 1.1 Schematic illustration of matters at relevant scales ranging from 1 Å to 10 m and the resolution range for the human eye, optical microscopy and electron microscopy.

Nanotechnology in Medicine

The definition of nanotechnology is manipulation of matter on an atomic, molecular, and supramolecular scale. Nanotechnology has contributed with powerful tools within nanomaterial design and resulted in a remarkable impact within medicine lately^{3,4}. Nanotechnology permits development of new types of nanomaterials for medicine with capabilities for dedicated targeting and drug delivery. These medical nanomaterials can be designed with multiple functionalities allowing therapeutic and diagnostic capabilities into one single *theragnostic* agent. Theragnostic agents enable the possibility to monitor treatment response and thereby personalize the medical procedure. This concept of *personalized medicine* is under way and has potential to eliminate or reduce side-effects from the standard medical treatments used today^{5,6}.

Novel nanomaterials intended for medical purposes are required to be biocompatible i.e. safe by design, and there are several aspects that need to be investigated prior to application. The size, shape and structure of the material must be controlled and maintained *in vitro* and *in vivo*, since the properties of a material are typically size-dependent in the nanoscale region, which requires that the material possesses colloidal stability in adequate media. Size and size distribution of nanomaterials as contrast agents are of main importance. There is a strict upper limit for nanoprobe designed to be cleared out through the kidney. In addition, the size and shape of nanomaterials are influencing the cellular delivery, blood-brain barrier passage and efficiency for renal excretion, which has a cut-off size limit $< 10\text{ nm}$ ⁷⁻⁹. Capping or coating with organic, biocompatible materials can be useful to provide colloidal stability, protect the surface properties of the nanomaterial, reduce adsorption of proteins and further functionalizations¹⁰. The adsorption process of proteins is typically called protein corona formation and it plays a key role in the biological fate of a nanomaterial introduced *in vivo*¹¹. Careful characterization and design are of high importance in the development of novel medical nanomaterials¹².

Rare Earth Elements

The rare earth (RE) elements include all the lanthanoids (La 57- Lu 71), scandium (Sc 21) and yttrium (Y 39)¹³ and are usually grouped together since they have similar ionic radii and they tend to occur in the same ore. The name RE originates from that the elements are naturally dispersed and occur in low concentrations in minerals. The RE elements display similar chemical properties and are strongly electropositive, typically existing in their trivalent state (some lanthanoids can form additional oxidation states)¹⁴. The radii of the trivalent lanthanoid ions are successively decreasing upon the atomic number, *lanthanoid contraction*¹⁵. This contraction and the sequential filling of the shielded 4f-orbital from cerium (Ce 58) to lutetium (Lu 71) is responsible for the unique electronic, optical and magnetic properties among the RE elements¹⁶.

Cerium

Cerium (Ce 58) is a lanthanoid (or lanthanide) and a RE element that has a crustal abundance about 66 mg/kg, which is similar to the abundance of copper (Cu 29)¹⁷. Cerium has the electronic configurations of $[\text{Xe}]4f^15d^16s^2$ as atom and $[\text{Xe}]4f^1$ as trivalent ion¹⁸. The chemistry of cerium stands out among the RE elements, since it can form stable compounds in oxidation state 4+ when it has the electronic configuration of the noble gas xenon (Xe 54)¹⁴. Cerium reacts easily with oxygen and forms either Ce_2O_3 or CeO_2 dependent on temperature and oxygen pressure, where the dioxide structure is preferable under normal temperature and pressure. CeO_2 has a cubic fluorite structure where each cerium cation is coordinated by eight oxygen anions. The presence of oxygen vacancies are normally found in the cerium oxide structure, therefore cerium oxide compounds have a stoichiometric value that differs from CeO_2 and with mixed valence states of Ce^{3+} and Ce^{4+} ^{19,20}.

Cerium oxide has a unique redox chemistry originating from feasible interconversion between Ce^{3+} and Ce^{4+} and the material has been applicable in heterogeneous catalysis, water-gas-shift reactions and solid-oxide fuel cells²¹. Scaling down cerium oxide crystals to nano-dimensions deviate from the stoichiometric value of CeO_2 , resulting in increase of the $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio²² and fundamentally enlarge the surface to volume ratio. These phenomena can together enhance the redox activity of cerium oxide^{23,24}.

Cerium Oxide Nanoparticles & Antioxidant Properties

Recently, the redox properties of cerium oxide nanoparticles have been found to mimic antioxidant behavior, e.g. scavenging of reactive oxygen species (ROS)²⁵. ROS are reduced metabolites of O_2 , such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) which are traditionally regarded as toxic bi-products of cellular metabolism with the potential to damage lipids, proteins and DNA²⁶. The damaging effects of ROS are prohibited by antioxidant enzymes such as superoxide dismutase (SOD, which reduces O_2^- to H_2O_2), catalase and glutathione peroxidase (which reduces H_2O_2 to H_2O)²⁷. In addition to the well-known antimicrobial effects, ROS have emerged to be important cell signaling molecules in several essential physiological processes such as cell migration, circadian rhythm and stem cell proliferation²⁸. The ROS balance is important to maintain since insufficient antioxidant capacity will result in oxidative injuries of cell components (oxidative stress) whereas an excess in antioxidant capacity will disturb cell signal transductions.

Cerium oxide nanoparticles have regenerative antioxidant capabilities due to the feasible interconversion between Ce^{3+} and Ce^{4+} ²⁹ and scavenge multiple ROS. They are proposed to possess activities like antioxidant enzymes such as SOD and catalase³⁰⁻³². SOD exists in metal-coordinated structures (Cu (n=1), Mn (n=2), Fe (n=2) and Ni (n=2)) and catalyzes the conversion of superoxide to H_2O_2 and molecular oxygen, as presented in Equation 1.1 and Equation 1.2³³.

Catalases are antioxidant enzymes that catalyze the conversion of H_2O_2 to water and molecular oxygen. The catalase mechanism is not fully understood, however a simplified reaction mechanism approximation is given in Equation 1.3 and Equation 1.4 ³⁴.

The antioxidant mechanisms of cerium oxide nanoparticles are under investigation. Celardo et al proposed a molecular mechanism for the SOD and the catalase activities of cerium oxide nanoparticles³⁵.

The proposed SOD mechanism is presented in Figure 1.2 and starting from sequence 1-2 where a superoxide binds to an oxygen vacancy site close to two Ce^{3+} . Thereafter there occurs an electron transfer from one Ce^{3+} to one oxygen atom. This enables a reaction between two surface-bounded electronegative oxygen atoms and two hydrogen atoms (protons) in the solution, resulting in a molecule of H_2O_2 (3). The H_2O_2 is released from the surface and the resulted oxygen vacancy site is open for a second superoxide to attach (4). This superoxide may oxidize another Ce^{3+} and further on react with two new protons from the solution. A second molecule of H_2O_2 is formed and released. The initial oxygen vacancy site with Ce^{3+} is oxidized (5) and the vacancy site with two Ce^{4+} may act as a binding site for a H_2O_2 (6). The H_2O_2 is oxidized, resulting in the release of two protons (7), one oxygen molecule and the oxygen vacancy site is restored with two Ce^{3+} .

The proposed catalase activity of cerium oxide nanoparticles is presented in Figure 1.3 and sequence 1-4 is identical to the oxidative half-reaction in Figure 1.2 (sequence 5-1). At sequence 5 in Figure 1.3, a second H_2O_2 binds to the oxygen vacancy site with two Ce^{3+} , followed by a reaction with uptake of two protons, homolysis of the O-O bond and transfer of electrons to the two Ce^{3+} at step 6. Two water molecules are formed and they are released from the oxygen vacancy site, resulting in an oxygen vacancy site with two Ce^{4+} (1).

Figure 1.2 A proposed reaction mechanism for the disproportionation reaction of superoxide on a nanoparticle surface of cerium oxide, redrawn from Celardo et al³⁵.

Figure 1.3 A proposed reaction mechanism for the disproportionation reaction of H_2O_2 on a nanoparticle surface of cerium oxide, redrawn from Celardo et al³⁵.

Oxidative stress is considered to be involved in the progress of many neurodegenerative diseases³⁶ and cerium oxide nanoparticles have shown promising capabilities to be neuroprotective^{37,38}. Zhou et al showed that cerium oxide nanoparticles prevent age-related macular degeneration *in vivo*³⁹. However, the health effects of cerium oxide nanoparticles are debated⁴⁰ and cytotoxic effects have been observed. Hussain et al presented mitochondrial-mediated apoptosis in human monocytes treated with cerium oxide nanoparticles⁴¹. Cheng et al showed that localization of cerium oxide nanoparticles inside lysosomes induced damage and apoptosis in human hepatoma cells⁴² and cerium oxide nanoparticles have been shown to be cytotoxic in cancer cell lines^{43,44}. The antioxidant properties of cerium oxide nanoparticles is related to the surrounding pH⁴⁵ and Asati et al demonstrated specific cytotoxic behavior of cerium oxide nanoparticles when they were internalized in acidic environments such as inside lysosomes or cancer cells⁴⁶. This change from antioxidant in normal pH to cytotoxic in acidic pH environment have shown promising results for cerium oxide nanoparticles to being utilized as an anti-cancer drug itself⁴⁷ and with radiation, it has been shown that cerium oxide nanoparticles radio sensitize cancer cells while protecting cells with normal (about neutral) pH⁴⁸.

Gadolinium

Gadolinium (Gd 64) is a lanthanoid and a RE element that has a crustal abundance of 6.2 mg/kg¹⁷. Gadolinium has the electronic configurations $[\text{Xe}]4f^75d^16s^2$ as atom and $[\text{Xe}]4f^7$ as trivalent ion^{14,18}. The trivalent gadolinium ion can organize its seven 4f electrons in the half-filled shell with parallel spins, giving Gd^{3+} largest possible total spin ($S=7/2$) and thereby a very large spin magnetic moment. The symmetric electronic structure provide a slow electronic relaxation rate. The large magnetic moment, the slow electronic relaxation rate and paramagnetic properties at room temperature make gadolinium highly useful for contrast enhancement in magnetic resonance imaging (MRI)⁴⁹⁻⁵¹.

Magnetic Resonance Imaging

MRI is a medical application of nuclear magnetic resonance and was developed by Paul Lauterbur and Sir Peter Mansfield, who together shared the Nobel Prize in Medicine in 2003. Today, MRI is routinely used at clinics all over the world to form images of anatomy and physiological processes of the soft tissues and is an excellent complement to X-ray or Computed Tomography (CT) examinations^{52,53}.

Figure 1.4 MRI is routinely used in clinics to create images of tissues in the body. This picture is from the presentation of the Nobel Prize in Physiology or Medicine 2003 at nobelprize.org and is here used by permission of The Nobel Committee for Physiology or Medicine (copyright).

Nuclear magnetic resonance is based on the phenomenon where magnetic nuclei of atoms exposed to an external magnetic field can absorb energy from irradiation of a certain radio frequency. This absorption process results in a change or flip of the magnetic nuclei's orientation⁵⁴⁻⁵⁶. When a magnetic field is applied, the randomly oriented magnetic moments or spins of the nuclei atoms align with the direction (z-axis) of the external magnetic field. The spins are wobbling around the z-axis at a rate referred to as the *Larmor frequency* and this wobbling motion is termed *precession*. The Larmor frequency can be calculated from knowing the gyromagnetic ratio of the nuclei and the magnitude of the applied magnetic field. Protons (^1H) are typically used for creating images in MRI, since hydrogen atoms are naturally abundant in biological organisms, particularly in water and fat. The Larmor frequency of a proton in a 1.5 T magnetic field is 63.9 MHz. When the individual spins are settled into a stable state, the spins will be oriented parallel or anti-parallel with the applied magnetic field. The number of parallel spins are slightly predominant and they build up a cumulative net magnetic vector (NMV) in the z-direction, *longitudinal magnetization* M_z (Figure 1.5). An applied 90° radio frequency pulse matching the Larmor frequency can excite the spin system and flip the NMV 90° from the z-axis into the xy-plane. The resulting magnetization is now in the xy-plane and is referred to as *transverse magnetization*, M_{xy} . The precession or the magnetic rotational motion in the xy-plane induces an alternating voltage in a receiver coil, which is the magnetic resonance (MR) signal. The MR signal is at maximum in the presence of transverse magnetization and is collected by several sensitive receiver coils⁵⁷.

Figure 1.5 This schematic drawing of magnetic spins that are randomly oriented and can (in this simplified picture) be organized parallel (or anti-parallel) with an applied external magnetic field of magnitude B_0 . The predominant parallel spins result in a cumulative net magnetization vector (NMV) in the same direction as the applied magnetic field. There are also magnetic vectors in x- and y-direction due to the precessional paths of the spins (not showed in this drawing), see Heisenberg's uncertainty principle.

Transverse magnetization rapidly fades and the spins realign with z-axis of the external magnetic field due to two processes, *spin-lattice relaxation* and *spin-spin relaxation* (presented in Figure 1.6). Spin-lattice relaxation (also called longitudinal or T_1 relaxation) is the regrowth of net magnetization along the z-axis. This relaxation process requires dissipation of energy from the spin system to its surrounding (lattice). Spin-spin relaxation (also called transverse or T_2 relaxation) is the loss of transverse magnetization due to the dephasing process. After excitation, the individual spins are coherent in phase. This phase coherency will be gradually lost as the individual spins advance in different directions and at different rates in their precessional paths. At some point, the individual magnetization vectors in the xy-plane will cancel each other out and then T_2 relaxation is completed. The times it takes for spin-lattice and spin-spin relaxation to take place are respectively denoted as T_1 and T_2 . T_1 and T_2 relaxations are two simultaneously processes that are independent of each other. The contrast in a MR image of a biological tissue depends on T_1 , T_2 and the proton density. Proton density is related to the water and fat content in the tissue and it means the number of excitable spins per unit volume⁵⁷.

Figure 1.6 a) The net magnetization vector of the spin system is parallel with the applied external magnetic field along z-axis, longitudinal magnetization M_z . A 90° pulse, with the same frequency as the Larmor frequency, b) flip the magnetization to the xy-plane and c) the spins precess around the z-axis. d) During relaxation the individual magnetic vectors (green) precess at different rates and in different directions. Blue vectors are projections of the green vectors in the xy-plane and in e) the projected magnetic vectors are cancelling each other out, thereby T_2 relaxation is finished. T_1 relaxation is finished in f) when the net magnetic vector has realigned with the z-axis.

Measuring T_1 and T_2

T_1 and T_2 can be measured by a number of pulse sequences and the commonly used inversion recovery and spin echo pulse sequences have been utilized in this thesis. The inversion recovery sequence is measuring T_1 by applying a 180° pulse that flips the magnetization vector of the spin system into the negative z-axis direction. The longitudinal magnetization is indirectly measured by applying 90° pulses at a number of time delays after excitation (180° pulse). A mathematical curve is fitted to these measurement points and it yields T_1 ⁵⁸.

T_2 is measured by utilizing a spin echo pulse sequence, which is initiated with a 90° pulse, flipping the magnetization to the xy-plane. The spins start to dephase and the transverse magnetization decreases. The individual proton magnetization vectors have to be recombined in order to be measurable in the receiver coil. This can be done by applying a 180° pulse at time τ and measure the MR signal at the echo time 2τ . The echo time is defined as the time between the 90° pulse and the time for the completion of rephasing. However, the rephasing is not completely fulfilled, since the dephasing process is a result from molecular interactions. The spin echo signal sample procedure is repeated multiple times and the monitored transverse magnetization will decrease successively as a result of T_2 relaxation. The magnitude of the transverse magnetization will be plotted against the measured echo times and a mathematically fitted curve yields T_2 ⁵⁹.

MRI Contrast Agents

MRI can only distinguish specific anatomy or physiological processes, which possess different characteristics such as T_1 , T_2 or proton density than its surrounding tissues⁶⁰. Contrast agents can significantly shorten T_1 and T_2 relaxation times on the specific target localizations and hence have a significant role in improving image quality in MRI. Contrast agents are used in about 25 % of all MRI examinations⁶¹ and the gadolinium-based contrast agents (GBCAs) are predominantly used⁶². The GBCAs are chelates, meaning that organic ligands are coordinated to the Gd^{3+} and stabilize the metal ion. These agents are either linear or macrocyclic chelates depending on the structure of the coordinated organic ligands⁶¹. Free Gd^{3+} ions are toxic in biological systems, since it competes with Ca^{2+} and when bound to a Ca^{2+} -binding enzymes, it may alter the biological processes catalyzed by these enzymes⁶³. A decade ago, it was found that the rare skin disease Nephrogenic Systemic Fibrosis (NSF) was developed by patients who had renal impairment and were administered linear GBCAs. The development of NSF was linked to the depositions of gadolinium⁶⁴⁻⁶⁶. Precautions in the administration of GBCA to patients with renal impairment were taken and subsequently the cases of NSF have substantially decreased⁶⁷. The MRI signal is in general low, and usually patients have to be administered large quantities of a contrast agent, often on the gram scale, to obtain adequate images. Recently, there have been concerns about the safety of GBCAs derived from Gd^{3+} ion release from the complexes (especially the linear agents) *in vivo* and the large quantities required for a successful MRI scan⁶⁸. There is an urgent need to

develop new safer contrast agents with improved relaxivity rates which could reduce the quantities of gadolinium in clinical use.

GBCAs are primarily used in T_1 -weighted MRI, and the intrinsic capability of a contrast agent to shorten T_1 is referred to as T_1 -relaxivity. There are several parameters influencing T_1 -relaxivity. The major ones are Gd^{3+} - 1H distance, hydration number, water exchange rate and rotational diffusion (in magnetic fields above 1.5 T). The T_1 -relaxivity (and also T_2 -relaxivity) of gadolinium is based on the dipolar interactions between the protons and the 4f electrons of gadolinium. The strength of this interaction is inversely proportional to the sixth of power to the distance (d^{-6}). A higher number of water molecules that can coordinate to Gd^{3+} (e.g. hydration number) improves relaxivity and can be achieved by reducing the number of coordinating ligands. However, the coordinated ligands stabilize the dispersibility and prevent the release of Gd^{3+} . A reduction of the number of ligands may cause instability of the contrast agent (chelate). The exchange of coordinated water molecules (e.g. water exchange) must be rapid in order to transmit the relaxation effect of Gd^{3+} to the solvent, but not too fast because the water is not coordinated to Gd^{3+} long enough to be relaxed. Slowing down the rotational time can at magnetic field strengths above 1.5 T can significantly improve the T_1 -relaxivity⁶⁸⁻⁷⁰. The most common used GBCAs in clinic display relaxation rates about $3-5 \text{ mM}^{-1}\text{s}^{-1}$ ⁶¹. Previously, nanoparticles of gadolinium oxide have shown to increase the T_1 -relaxivity rate to about $6.9 \text{ mM}^{-1}\text{s}^{-1}$ ⁷¹. Park et al has demonstrated a correlation between T_1 -relaxivity rate and the number of surface gadolinium atoms, there the T_1 -relaxivity was highest for gadolinium oxide nanoparticles with sizes about 1-2.5 nm⁷². Gd-based nanoparticles also have the advantage of bringing many gadolinium atoms into one entity, thereby they will deliver a greater cumulative signal in MRI per entity (enhanced local contrast) compared to the Gd-based chelates⁷³.

Useful terms in MRI

T_1 is a relaxation mechanism also termed as spin-lattice or longitudinal relaxation. In addition is T_1 is the time for a spin-lattice relaxation to occur, unit s (seconds).

T_2 is a relaxation mechanism also termed as spin-spin or transverse relaxation. The time for a spin-spin relaxation to occur is also abbreviated T_2 , unit s.

$R_1=1/T_1$ and is a value of the rate for a T_1 -relaxation, unit s^{-1} .

$R_2=1/T_2$ and is a value of the rate for a T_2 -relaxation, unit s^{-1} .

r_1 is the ability for a contrast agent to change R_1 , unit $\text{mM}^{-1}\text{s}^{-1}$.

r_2 is the ability for a contrast agent to change R_2 , unit $\text{mM}^{-1}\text{s}^{-1}$.

Relaxivity is a general term of a contrast agent's ability to affect water molecules to undergo relaxation.

Research Objectives

The overall aim is to utilize cerium oxide nanoparticles for guiding the MR contrast enhancement properties of gadolinium to a target site and on-site milder eventual inflammation. Following four research objectives represent the process of this thesis work.

1. Prepare cerium oxide nanoparticles with defined compositions of gadolinium and characterize the as-prepared nanoparticles according to size, shape and crystal structure.
2. Investigate the antioxidant and MR contrast enhancing properties of the cerium oxide nanoparticles with a full set of gadolinium integrations.
3. Prepare dextran-coated gadolinium-cerium oxide nanoparticles and investigate the size, surface charge and the antioxidant and MR contrast enhancing properties.
4. Investigate protein corona formation on gadolinium-cerium oxide nanoparticles prepared with and without surface coating of dextran.

2. Synthesis & Methods

Synthesis

In this thesis work, cerium oxide nanoparticles with integrated gadolinium have been synthesized by a precipitation synthetic route. This is a simple solution based approach that has been commonly used for constructing cerium oxide nanoparticles with controlled size, shape and physiochemical properties^{45,74-76}. Cerium and gadolinium either acetate or nitrate salts have been mixed at gadolinium/cerium ratios between 0 and 1. These salts have been mixed in a solution consisting of water and an organic molecule, i.e. triethylene glycol (TEG), diethylene glycol (DEG) or dextran. The cerium/gadolinium composite oxides were then precipitated in the presence of ammonia under stirring conditions (no heat treatment). After 2 hours the synthesis was completed. The synthesized samples were then purified utilizing centrifugation and dialysis.

Sample name

The synthesized nanoparticles were named like CeO_x:Gd--%, where the molar percentage number corresponds to the measured composition of gadolinium. The Gd-ratio were calculated according to Equation 2.1 and the RE element concentrations were determined utilizing inductively coupled plasma mass spectroscopy (ICP-MS).

Equation 2.1
$$Gd - ratio = \frac{[Gd]}{[Ce] + [Gd]}$$

The sample names of the dextran-coated nanoparticles were of the same style but added @D or @FD in the end. These ending abbreviations were attributed to if fluorescein isothiocyanate (FITC) was conjugated to the dextran (@FD) or not (@D)

Dextran

Dextran is referred to a group of polysaccharides derived from the condensation of glucose and can be of varying chain lengths. They are used in a range of medical applications⁷⁷ and is commonly utilized for biocompatible coatings of nanoparticles intended for biomedical applications^{78,79}. Dextran-coated iron oxide nanoparticles for MRI have been approved by U.S Food and Drug Administration⁸⁰ and surface-grafted dextran is known to prevent protein adsorption^{79,81}. The utilized dextran molecules varied by molecular weights (in the range of 2000-5000 g/mol) and were either unconjugated or conjugated with fluorescein isothiocyanate (FITC). Dextran can be readily functionalized with amine groups and thereby enable a platform for further functionalities, such as targeted drug delivery^{82,83}.

Methods

Relaxivity

T_1 and T_2 were determined with a relaxameter (1.41 T), utilizing an inversion recovery and a spin echo pulse sequence (Paper I, II, IV, VI). The relaxation times were measured for the concentration series of respectively synthesized nanoparticle samples. Thereafter, the inverse of T_1 and T_2 were plotted against the concentration of gadolinium. Each of the $1/T_1$ (R_1) and $1/T_2$ (R_2) data points were fitted with a linear function and the gradients correspond to the relaxivities r_1 and r_2 .

X-ray Diffraction

The crystal structure of $\text{CeO}_x\text{:Gd}$ nanoparticles were investigated utilizing powder X-ray diffraction (XRD) and these measurements were performed in Paper I and II. Powder of the synthesized nanoparticles were obtained by either rotary evaporation or liquid-freeze drying. The principle of XRD is described by *Bragg's law* given in Equation 2.2^{84,85}.

Equation 2.2
$$n\lambda = 2d_{hkl}\sin\theta$$

n is an integer, λ is the wavelength of the X-ray beam, d_{hkl} is the distance between the crystal plane with Miller index hkl (crystal orientation) and θ is the incident angle of the X-ray beam.

The procedure for the XRD-measurements was as follows. A monochromatic X-ray-beam was directed on the fine powder sample, which consist of randomly oriented crystallites. The crystallites scattered the incident X-rays and the diffraction was monitored by an X-ray detector. The diffractometer scanned over a range of angles, and when the angle fulfilled the Bragg's law (Bragg angle), constructive interference was occurring. This measurement resulted in a diffractogram, with intensity on y-axis and the scattering angle x-axis (scattering angle is two times the incident angle). The diffractogram provided crystal information and was used for material identification and estimations of lattice parameters. In nanoparticle samples, the peaks in the diffractogram will be broaden as a result of constructive interference at angles that slightly differ from the Bragg angle. This peak broadening is related to the product of distances for the crystal planes that produced a specific diffraction peak, and can be used for estimating the crystal or grain size with the Scherrer equation, Equation 2.3.

Equation 2.3
$$\text{Grain size} = \frac{K\lambda}{B(2\theta)\times\cos\theta}$$

K is the shape factor and 0.9 is a fair approximation on shape factor for spherical crystals of cubic structure, λ is the wavelength of the X-ray beam, $B(2\theta)$ is the peak width and the full-width at half maximum was used in this thesis work, and θ is the incident angle of the X-ray beam^{86,87}.

Dynamic Light Scattering

Dynamic light scattering (DLS) was utilized to measure the hydrodynamic diameter of the prepared nanoparticles (Paper I-V). DLS is based on scattering of light by diffusing particles undergoing Brownian motion. Larger particles undergo slower diffusion, resulting in slower fluctuations in the measured scattered light intensity. The fluctuations in intensities are input for the DLS analysis, handled by a correlator. A correlator compares the signal intensities at varying time intervals. At a short time interval, dt , the correlation between the signals at t and $t + dt$ will be very strong. If signals at $t+2dt$, $t+3dt$, $t+4dt$ etc. are compared with the signal at t , the correlation will be decreasing over time since the nanoparticles are moving randomly. Perfect correlation occurs when the compared signals are identical and it is indicated by 1, and no correlation is indicated by 0. Based on this, a correlation function $G(\tau)$ is constructed given in Equation 2.4, where τ = sample time for the correlator. This function is an exponentially decaying function of the correlator time delay τ .

Equation 2.4
$$G(\tau) = A[1 + B * \exp(-2\Gamma\tau)]$$

A = baseline of the correlation function. B = intercept of the correlation function. $\Gamma = Dq^2$. D = translational diffusion coefficient. In the case of spherical particles, Stoke-Einstein equation can be applied and give $D = k_B T / 6\pi\eta r$ (k_B = Boltzmann constant, T = temperature in Kelvin, η = viscosity of solvent and r = particle radius). $q = (4\pi n / \lambda_0) \sin(\theta/2)$ (n = refractive index of dispersant, λ_0 = wavelength of the laser and θ = scattering angle). For polydisperse samples the correlation function can be written as in Equation 2.5

Equation 2.5
$$G(\tau) = A[1 + B * g_1(\tau)^2]$$

$g_1(\tau)$ = sum of weighted exponential decays contained in the correlation function.

Size distributions of the measured sample can be obtained from the correlation function through two main algorithmic approaches, e.g. single exponential fit analysis and multiple exponential fit analysis. The single exponential fit analysis or *Cumulant analysis* is a polynomial fit to the semi-log plot of the correlation function. The fit can produce a number of values, where the two main terms are the mean value for the size (Z-average diameter) and the width parameter known as *polydispersity index* (PDI). The cumulant analysis is acceptable when the PDI is low ($\sim \text{PDI} < 0.1$) and then the sample can be considered as monodispersed.

For polydispersed sample, a multiple exponential has to be fitted to the correlation function, and *CONTIN* or *Non-negative least squares* (NNLS) are two methods to find calculate the multiple exponential fit. Both *CONTIN* and NNLS basis on the prior knowledge that distribution functions are represented by positive numbers. The *CONTIN* analysis has additional prior knowledge, the simplest solution is preferred (parsimony principle). Thereby, *CONTIN* partly overcomes the dependency on the range of particle size sets, which is one of the main weak points for NNLS. One drawback with *CONTIN* is that the final results are very sensitive to small differences in baseline estimates. The results from *CONTIN* (or NNLS) is known as *intensity size distribution* and is a plot of the relative intensity of light scattered by particles in various size classes

If the intensity size distribution plot shows more than one peak or there is a substantial tail of a peak, an algorithm based on *Mie theory* can be used to convert the intensity distribution to a volume or a number distribution. *Mie theory* describes the scattering of an electromagnetic plane wave by a homogenous sphere. According to *Rayleigh approximation*, the probability for scattering is proportional to d^6 (d = particle diameter). Meaning that 1 particle with a diameter of 100 nm scatters 10^6 higher light intensity compared to a particle with a diameter of 10 nm. The algorithm, converting an intensity distribution to a number distribution, is very often useful to give a more realistic view of the sample⁸⁸⁻⁹⁰.

Zeta Potential

The Zeta potential of the synthesized nanoparticles in solution were measured in Paper I-V. The surface charge of nanoparticles in a solution will be electrically neutralized by ions in the fluid, forming an electrical double layer. The first electrical layer closest to the particle surface consists of a stationary layer of fluid attached to the particle and the outer layer is a continuously exchangeable fluid layer. The interface between the stationary and the exchangeable fluid layers is defined as the slipping plane. Zeta potential is defined as the potential difference between the slipping plane and the dispersion medium. Zeta potential is the potential of practical interest in dispersion stability since it determines the interparticle forces.

Zeta potential is indirectly measured using laser Doppler electrophoresis (or even called laser Doppler velocimetry). This technique is based on electrophoresis, which is a phenomenon where an electric field is applied across the dispersion and the dispersed, charged particles will move toward the electrode of opposite charge. By using a laser and measure the scattered light from the dispersed particles, a frequency shift can be observed and measured when the sample is undergoing electrophoresis. The measured frequency shift and given values of the laser wavelength and the scattering angle is used for determining the electrophoretic mobility, e.g. the velocity of the particles in an electrical field. Thereafter, an equation based on the theory of Smoluchowski can give the Zeta potential of particles in polar media^{91,92}.

Electron Microscopy

Spatial resolution refers to the smallest distance between two points where they both can be resolved. There are several parameters determining the resolving power for an imaging modality. Primarily, the wavelength of the source of illumination is the most important limitation in spatial resolution and the Rayleigh criterion states the smallest distance δ that approximately can be resolved, Equation 2.6.

Equation 2.6
$$\delta = \frac{0.61\lambda}{NA}$$

λ is the wavelength of the radiation and NA is the numerical aperture of the optical system in the imaging modality. This limits the resolution of optical microscopy to approximately 200 nm.

Electrons are utilized as the illumination source in electron microscopy and they can be accelerated to wavelengths smaller than 0.004 nm at operation voltages above 100 keV. However, due to instrument limitations the effective resolution is ~ 0.1 nm, which is still comparable to the general atomic radius. An electron beam is a type of ionizing radiation, since it has the capability of separating inner-shell electrons from their respective nuclei. This means that the incident electrons transfer energy to individual atoms in the specimen, resulting in a range of secondary signals shown in Figure 2.1.

Figure 2.1 When a high energy electron beam interacts with a specimen several phenomena such as scattering, absorption and photoelectric effect occurs. These phenomena result in various signals that can be detected in different types of electron microscopy. The directions of the illustrated signals are indicating (in a relative manner), where the signal is strongest and where it is detected. This Figure is redrawn from Williams et al⁹³.

There are two main types of electron microscopy, scanning (SEM) and transmission (TEM). SEM produces images of a sample by scanning the surface with a focused electron beam and detecting either backscattered or secondary electrons. The spatial resolution is approximately 1 nm or finer for the SEM. The SEM-samples are usually sputtered with a thin conductive layer, for example a few nm thick platina layer. TEM is based on the detection of inelastically scattered electrons as they travel through and interact with a thin sample, <100 nm. These transmitted electrons are then focused and magnified in order to form an image, using much the same principle as in optical microscopy. Scanning transmission electron microscopy (STEM) also scans the specimen but produce images as conventional TEM (Paper I, IV and VI). Today, electron microscopies can produce images with atomic resolution^{93,94}.

Secondary signals are used in analytical electron microscopy to provide chemical information about the studied sample. Energy Dispersive X-Ray Spectroscopy (EDX) and Electron Energy Loss Spectroscopy (EELS) are two common techniques in analytical electron microscopy and have been utilized in this thesis in conjunction with studies using Scanning Electron Microscopy (SEM, Paper V)) and Scanning Transmission Electron Microscopy (STEM, Paper II). EDX measures the energy of X-rays emitted from the surface as a result of outer shell electrons relaxing to fill the hole created by the emitted inner shell electron. Whilst EELS measures the energy transferred from the incident to the inner shell electrons

STEM-EELS was utilized to measure the electronic configurations on small regions (6.5x6.5 Å²) of CeO_x, CeO_x:Gd9% and CeO_x:Gd19% (Paper II). The regions were either defined as core or surface according to the example in Figure 2.2. EELS spectra with the range of 500-1250 eV were obtained for several regions to improve the signal to noise ratio. These studies were done in collaboration with Professor Per Persson, Thinfilm Physics, Linköping University, who performed the measurements and analysis.

Figure 2.2 STEM-EELS enable study of electronical configurations on small regions at the surface and at the core of individual nanoparticles.

Atomic Force Microscopy

Atomic force microscopy (AFM) is a scanning probe microscopy technique that can provide images with spatial resolution in the nanoscale. AFM in tapping mode was used for image a neutrophil (Paper V). In tapping mode, a tip is connected to a cantilever, which is oscillating (close to its resonance frequency) over a sample. Forces such as Van der Waals forces, dipole-dipole and electrostatic forces will interact between the tip and the sample. When the tip is approaching the sample surface, the amplitude of the oscillating cantilever will change. The amplitude is an input parameter to an electronic servo (typically a piezo element) that controls the height of the cantilever. The height will be adjusted to maintain the set oscillating amplitude of the cantilever. The adjustment in height will be recorded and the resulting image will be representing the topography of the sample⁹⁵.

Optical Spectroscopy

Optical spectroscopy studies absorption and emissions of light by matter and the principle can be described with a Jablonski diagram, drawn in Figure 2.3. Absorption in the ultraviolet and visible regions (UV-VIS) of $\text{CeO}_x\text{:Gd}$ nanoparticles treated with H_2O_2 in Paper II. Absorption of light followed by emission of light is termed fluorescence. Fluorescence studies were employed for the $\text{CeO}_x\text{:Gd@FD}$ nanoparticles (Paper IV) and in the performed biological assays, described later in this chapter⁹⁶.

Figure 2.3 A Jablonski diagram showing the possible energy transitions after a molecule has been photoexcited. Fluorescence and phosphorescence are radiative transitions. Non-radiative transitions are presented as undulating arrows.

X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) is a surface sensitive technique, providing information of the elemental composition of a solid's outer surface, within the first 10 nm. It is a user-friendly technique, which has the abilities to 1) identify and quantify the elemental composition and 2) reveal the chemical environment where respectively element exist in. XPS is based on the photoelectric effect, which is a physical phenomenon where electrons (or other free carriers) are emitted upon irradiation of light, typically ultraviolet light or X-rays. The emitted electrons in XPS are called photoelectrons and they are given a kinetic energy (E_k) according to Equation 2.7.

Equation 2.7
$$E_k = E_{ph} - \Phi_{XPS} - E_B$$

E_{ph} is the energy of the incident photons, Φ_{XPS} is the work function of the instrument and E_b is the binding energy for the emitted electrons.

Usually, monochromatic X-rays of Al K α or Mg K α are used for irradiating a sample in XPS. This results in the emission of photoelectrons with kinetic energies dependent on their respectively binding energy. Thereafter, the emitted electrons are analyzed in an energy analyzer, used as a filter, only allowing photoelectrons with a specific kinetic energy to be detected. The intensity of emitted electrons for respectively kinetic energy is recorded and a spectra is obtained. The binding energies of the emitted electrons can be calculated utilizing Equation 2.7, since E_{ph} and Φ_{XPS} are known values⁹⁷. The binding energies are unique for each element, which makes XPS suitable for elemental composition. Chemical states can also be obtained based on analysis of chemical shifts. In this thesis, XPS was used for determining the composition of oxidation states in CeO_x:Gd nanoparticles treated with H₂O₂ (Paper II).

Near Edge X-ray Absorption Fine Structure

Near edge X-ray absorption fine structure (NEXAFS) is a type of absorption spectroscopy, providing information about the electronical and chemical structure of a sample. The incident light is swept over an absorption edge of an element and photoemitted electrons, inelastically scattered photoelectrons, auger electrons and fluorescent photons can be detected (Total Electron Yield detection mode)⁹⁸. NEXAFS spectra of Ce³⁺ and Ce⁴⁺ were experimentally measured and theoretically calculated in Paper I.

Infrared Spectroscopy

Infrared (IR) spectroscopy is a method for studying chemical structures of molecules and the presence of functional groups (Paper IV). The method is based on that molecules absorb IR light and are excited to a higher vibrational energy state. For IR-active molecules, it is required that the vibrational change has to cause a change in its electric dipole moment⁹⁹.

Raman Spectroscopy

Raman spectroscopy is in similarity with IR spectroscopy, a method for obtaining information of the chemical structures of molecules from their vibrational transitions (Paper II). In Raman spectroscopy the change in vibrational motion has to involve a change in polarizability¹⁰⁰.

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was used to study the desorption process of organic molecules surrounding the prepared CeO_x/Gd nanoparticles (Paper IV). The mass of the samples was measured over time as the temperature changed.

Biological Assays

In this thesis work, many biological assays have been performed thanks to cross-disciplinary collaborations. The assays will be shortly presented in this section.

The performed antioxidant and the cell viability assays have been based on the fluorescence methodology, which has been proven to provide real-time measurements of ROS in vitro and in vivo with high sensitivity and simplicity in data collection^{101,102}. Today there is a range of available fluorescent probes with different properties in aspects of sensitivity, specificity, localization, excitation and emission, and more probes are under development¹⁰³.

Three types of antioxidant assays have been performed; 1) Measured ROS production in human neutrophil granulocytes (neutrophils) utilizing a dichlorofluorescein (DCF) probe (Paper I and VI), 2) Luminol-based enhanced chemiluminescence of ROS in whole blood (Paper V) and 3) Luminol-based enhanced chemiluminescence of ROS in mice (Paper II). The DCF probe is a commonly used probe reacting with several types of ROS and its fluorescence signal is suitable as a marker for the total or general ROS-production¹⁰⁴. Neutrophils are the most abundant white blood cell in the blood circulation and their main role is to capture and destroy invading pathogens through several cellular mechanisms as for example production and release of ROS and various inflammatory mediators¹⁰⁵. In vitro, the release of ROS can be triggered by phorbol myristate acetate (PMA) via protein kinase C activation. In the first antioxidant assay, PMA was used for induction of ROS-production and the increasing ROS concentrations have been monitored with the DCF probe in the presence and absence of gadolinium-cerium oxide nanoparticles.

Luminol was used in the other two antioxidant studies and it is in similarity with DCF, a commonly used probe for studying the total ROS-production *in vitro* and *in vivo*^{106,107}. In Paper II, ROS-production in blood was initiated by a secondary activation of neutrophils and monocytes. The concentrations of ROS was measured with and without gadolinium-cerium oxide nanoparticles present using luminol. Monocytes are white blood cells that have multiple roles in the innate immune system and as neutrophils, they can also produce ROS¹⁰⁸. In the *in vivo* antioxidant assay, polystyrene microparticles were used for inducing ROS and the bioluminescence of luminol was monitored for several days in an immunocompetent mice model^{106,109}. In contrast to the performed *in vitro* assays, the long-term antioxidant effects of the gadolinium-cerium oxide nanoparticles could be examined.

Cell viability of neutrophils treated with gadolinium-cerium oxide nanoparticles was studied using PrestoBlue (Paper I and VI). PrestoBlue is a resazurin based reagent that is reduced in cellular respiration and thereby it changes from a non-fluorescent form to strong fluorescent form. The incubation time can be as short as 10 min and Xu et al showed in their study that the PrestoBlue assay has higher sensitivity than the well-known MTT assay¹¹⁰. A long-term viability assay was performed with human foreskin fibroblast exposed to gadolinium-cerium oxide nanoparticles (Paper I). The cytotoxic effects were evaluated after 7 days of exposure, utilizing the established crystal violet assay¹¹¹.

In conjunction with the antioxidant assay in blood (Paper V), the primary hemostasis was studied in the presence of prepared gadolinium-cerium oxide nanoparticles. Hemostasis is a biological process that causes a bleeding to stop and it can be represented by studying the activation of blood platelets¹¹². The platelet aggregation was induced by the snake venom convulxin¹¹³ and monitored using standard impedance and absorption measurements¹¹⁴.

In Paper III and IV, the protein corona formation of gadolinium-cerium oxide nanoparticles coated and uncoated with dextran, was investigated. The nanoparticles were incubated in human plasma for 1 h and thereafter the proteins were isolated and washed before they were identified utilizing 2D-gel electrophoresis, nanoflow liquid chromatography coupled to tandem mass spectrometry (nLC-MS/MS) and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF-MS). These three techniques are complementary in identifying the composition of the nanoparticle binding proteins^{115,116}. The identified proteins can contribute with information on the predicted fate *in vivo* of the studied nanoparticles¹¹⁷. The viability and cytokine release of monocytes treated with protein corona covered nanoparticles were investigated to dictate possible immune responses. The monocytes were exposed to these nanoparticles for 24 h and 72 h and the viability was assessed at 24 h utilizing the DNA binding agent SYTOXTM Blue¹¹⁸ and the cytokine release was measured with a well-known multiplex immunoassay (27-Plex Pro Human Cytokine kit)¹¹⁹.

3. Concluding Remarks and Key Results

In this chapter, the main results of the developed $\text{CeO}_x\text{:Gd}$ nanoparticles are summarized. The key results obtained from the characterization of the physical and chemical analysis are presented, followed by detailed biological experimental data. The intention with this section is to highlight for the reader our vision of this PhD project. The core results below represent a joint platform for the papers in this PhD thesis.

Material Characteristics

The performed XRD-patterns showed that the fabricated nanoparticles adopted the cubic fluoride structure typical for cerium dioxide. The XRD peaks were continuously broadened and shifted upon increased content of gadolinium (Figure 3.1 a, Paper II). Given by Scherrer equation and Bragg's law, the broadening was a result of smaller grain size and the shifting was a consequence of increased lattice parameter. Measurements of the hydrodynamic diameter with DLS and the Feret diameters of nanoparticles with TEM (Figure 3.2), confirmed that higher integration of gadolinium reduced the size of the nanoparticles. In addition, the aspect ratios (Figure 3.2 c) were increasing with a higher content of gadolinium, meaning that the particle shape became more elongated. Deshpande et. al. showed that the increased lattice parameter in smaller cerium oxide nanoparticles is related to the increased content of Ce^{3+} ²². This suggests, that increased integration of gadolinium will result in higher ratio of $\text{Ce}^{3+}/\text{Ce}^{4+}$, which was verified in the NEXAFS study (Paper I) and similar trends have also been reported previously¹²⁰. The Raman spectroscopy study is presented in Figure 3.1b (Paper II) and the results showed that integration of gadolinium affects the position and symmetry of the F2g-peak (cerium oxide) and that there was no observable peak attributed to the C-fold symmetry of gadolinium oxide at 362 cm^{-1} . The F2g-peak alterations can, according to Spanier et. al. be explained by introduction of more stress in the material²³. The absence of the peak at 362 cm^{-1} indicates that the prepared $\text{CeO}_x\text{:Gd}$ nanoparticles were of one single phase¹²¹. The STEM-images and the acquired STEM-EELS spectra for $\text{CeO}_x\text{:Gd}0\%$ (CeO_x), $\text{CeO}_x\text{:Gd}9\%$ and $\text{CeO}_x\text{:Gd}19\%$ indicates that the gadolinium atoms were homogeneously distributed in the nanoparticles. Thereby the STEM results are supporting the Raman result. The STEM-EELS data showed a 2% higher content of Ce^{3+} for surface regions compared to the core regions, for all the studied nanoparticles. To be noted, the core spectra were averaged with contributions from the surface regions, since the beam was transmitted through the particles.

Figure 3.1 a) XRD and b) Raman spectra of CeO_x:Gd0-19%.

Figure 3.2 Representative TEM-images of CeO_x, CeO_x:Gd9% and CeO_x:Gd19%. Feret diameters, minFeret diameters and aspect ratios were determined for at least 100 nanoparticles of respectively sample. This figure is redrawn from the original figure, which was published by Peter Eriksson in Scientific Reports¹²² and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

Coating of CeO_x:Gd with non- and FITC-conjugated dextran were successfully employed indicated by DLS-, Zeta potential, Attenuated Total Reflectance (ATR-IR), and TGA measurements (Paper IV). The DLS measurements showed an increase in hydrodynamic diameter for the dextran-coated nanoparticles (CeO_x:Gd@D/FD) compared to the as-prepared CeO_x:Gd. Furthermore, CeO_x:Gd@D/FD displayed similar

hydrodynamic diameters in MQ-water and cell media (RPMI 1640). The Zeta potentials were about neutral for the $\text{CeO}_x\text{:Gd@D/FD}$, suggesting that the dextran coating introduce steric hindrance and thereby capability for the nanoparticles to maintain dispersible in cell media. ATR-IR measurements contributed to identification of the organic molecules surrounding the prepared nanoparticles (Figure 3.3). The $\text{CeO}_x\text{:Gd}$ nanoparticles displayed molecular vibrations that can be attributed to acetate and DEG. Acetate was used as stabilizing ligand for the precursor and DEG as media in the synthesis, therefore were both of the organic molecules expected to be observed in ATR-IR data. The molecular vibrations of dextran were clearly present in all the $\text{CeO}_x\text{:Gd@D/FD}$ samples. The vibration nodes related to nitrate could not be noticed, meaning that dextran may have replaced the nitrate molecules on the surface. The TGA curves suggested a high grafting density of dextran. The nanoparticles with @FD - coating displayed emission at 518 nm (excitation 492 nm) and thereby fluorescent functionality was enabled for these developed $\text{CeO}_x\text{:Gd}$ nanoparticles.

Figure 3.3 ATR-IR measurement of prepared nanoparticles (CeO_x , $\text{CeO}_x\text{:Gd11\%}$, $\text{CeO}_x\text{@D}$, $\text{CeO}_x\text{:Gd13\%@D}$, $\text{CeO}_x\text{@FD}$ and $\text{CeO}_x\text{:Gd12\%@FD}$) and reference materials (DEG, Ce(III)acetate , $\text{Ce(III)nitrate hexahydrate}$, dextran (@D), FITC-conjugated Dextran (@FD))

Relaxivity

The $\text{CeO}_x\text{:Gd}$ nanoparticles had r_2/r_1 ratios about 2 or less, thereby they can be considered as T_1 -weighted contrast agents⁵⁰. In T_1 -weighted imaging the r_1 value is the most dominating parameter¹²³. The T_1 -relaxivities were ranging from $7.4 \text{ mM}^{-1}\text{s}^{-1}$ to $23.1 \text{ mM}^{-1}\text{s}^{-1}$ depending on the Gd-ratio and the surface coating (Paper I, II, IV and V). These r_1 values were about 2-6 times greater in magnitude compared the most common used GBCAs, which display relaxation rates about $3\text{--}5 \text{ mM}^{-1}\text{s}^{-1}$ ⁶¹. Higher r_1 values were obtained for $\text{CeO}_x\text{:Gd}$ nanoparticles with the lower content of gadolinium (Figure 3.4). $\text{CeO}_x\text{:Gd}5\%$ and $\text{CeO}_x\text{:Gd}9\%$ displayed approximately equal r_1 and therefore $\text{CeO}_x\text{:Gd}<10\%$ were considered to be most suitable for MRI applications. Dextran-coating could further improve the T_1 -weighted MRI contrast enhancement properties, there $\text{CeO}_x\text{:Gd}\sim 10\%\text{@D/FD}$ displayed r_1 above $20 \text{ mM}^{-1}\text{s}^{-1}$. This could be explained by a probable increase in rotational diffusion of the dextran-coated nanoparticles, since they had a larger hydrodynamic diameter than the as-prepared $\text{CeO}_x\text{:Gd}$ nanoparticles.

Figure 3.4 T_1 -relaxivities of $\text{CeO}_x\text{:Gd}5\text{--}19\%$ are given in the brackets and were obtained from the slopes of the linear curves fitted to the measured relaxation data.

Antioxidant and other biological properties

The antioxidant properties of CeO_x and $\text{CeO}_x\text{:Gd}$ nanoparticles originates in the capability of interconversion between Ce^{3+} and Ce^{4+} . Treating $\text{CeO}_x\text{:Gd}$ nanoparticles with H_2O_2 quickly resulted in a change from Ce^{3+} to Ce^{4+} , thus resulted in an absorbance shift and a color change as presented in Figure 3.5 a (Paper II). The color of the H_2O_2 exposed nanoparticles were slowly returning to its original color (Figure 3.5 b) and can then change color again upon addition of H_2O_2 (Figure 3.5 c). XPS was utilized for demonstrating the change in oxidation state of $\text{CeO}_x\text{:Gd}$ nanoparticles with the treatment of H_2O_2 (Figure 3.5 d-e). The relation between the shift in absorbance and antioxidant capacity has previously been shown by Lee et al²⁹ and Baldim et al¹²⁴.

Figure 3.5 Absorbance of untreated and treated $\text{CeO}_x\text{:Gd9\%}$ with H_2O_2 measured a) directly after addition, b) after 10 days and c) 10 days and a new addition of H_2O_2 . XPS of $\text{CeO}_x\text{:Gd9\%}$ a) untreated and b) treated with H_2O_2 .

The ROS-scavenging properties of the $\text{CeO}_x\text{:Gd}$ nanoparticles synthesized in the presence of TEG (Paper I) were studied in neutrophils utilizing the DCF probe. DCF intensity was traced for 1 h and it correlates to the general production of ROS¹⁰⁴. Controls (white bars) and the PMA-induced (grey bars) ROS-productions at 15 min and 45 min in the presence of the as-prepared $\text{CeO}_x\text{:Gd}$ nanoparticles are shown in Figure 3.6. All $\text{CeO}_x\text{:Gd}$ nanoparticles showed a modulatory effect on the ROS-production compared to the PMA-reference. The CeO_x nanoparticles displayed significant ROS-scavenging properties and did not induce any further generation of ROS compared to the control (without PMA). The $\text{CeO}_x\text{:Gd9-46\%}$ nanoparticles and especially $\text{CeO}_x\text{:Gd32-46\%}$ induced a minor ROS-productions, indicating a potential toxic effect of gadolinium. This was in agreement with a previous report, there non-coated gadolinium oxide nanoparticles (about 5 nm) induced ROS-generation while coating was shown to reduce induction of ROS¹²⁵. None of the prepared $\text{CeO}_x\text{:Gd}$ had any observed effect on the viability in neutrophils for 3 h (PrestoBlue assay).

Figure 3.6 ROS-production (DCF-intensity) of non-induced (white bars) and PMA-induced (grey bars) neutrophils at 15 min and 45 min in the presence of $\text{CeO}_x\text{:Gd}$ nanoparticles ($[\text{Ce}]=50 \mu\text{g/ml}$). $N=4-8$, minimum duplicate samples. ROS production (a.u) $\pm$ S.E.M. * = $P \leq 0.05$, ** = $P \leq 0.01$ (paired t-test, GraphPad Prism ver. 6.07). This figure was published by Peter Eriksson in Scientific Reports¹²² and is licensed under the Creative Commons attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).

The ROS-scavenging properties of CeO_x:Gd nanoparticles (synthesized in DEG) were also evaluated in a whole blood model (Paper V) and in an *in vivo* model (Paper IV) utilizing luminol amplified chemiluminescence. In the whole blood assay, ROS-production from neutrophils and monocytes was monitored following convulxin treatment. All the studied nanoparticle samples, CeO_x, CeO_x:Gd10% and CeO_x:Gd34%, displayed inhibition of ROS-production in blood and the inhibitions were approximately 50% for the highest dose ([Ce] = 30 µg/ml) of each CeO_x:Gd nanoparticles. Platelet activity was simultaneously monitored with a standard impedance measurement, and also in parallel evaluated in platelet rich plasma via light transmission. All studied CeO_x:Gd nanoparticles showed an inhibition of platelet aggregation, but was most pronounced for CeO_x:Gd34%. The long-term ROS-scavenging properties of CeO_x and CeO_x:Gd5% were investigated in an *in vivo* mice model. ROS-generation was induced by polystyrene microparticles. The generation of ROS were significantly lower on the sites of CeO_x and CeO_x:Gd5% nanoparticles compared to the control site. The inhibitory effects of the studied nanoparticles were persistent over the entire 5-day period of study, suggesting that CeO_x:Gd nanoparticles have long-term ROS-scavenging properties.

ROS-productions by neutrophils, in the presence of CeO_x (DEG), CeO_x:Gd11% (DEG), CeO_x@D and CeO_x:Gd13%@D nanoparticles, were monitored in during 1 h with DCF (Paper IV). PMA was used as a positive control inducing a potent generation of ROS. The ROS-productions at 15 and 45 min for all the studied concentrations ([Ce] = 10, 50 and 100 µg/ml) of the nanoparticles are shown in Figure 3.7 and Figure 3.8. All studied nanoparticles displayed an inhibitory effect of the ROS-production at 15 min, and the inhibitory effects were increasing with the concentration of cerium. At 45 min, the ROS-productions in the samples with CeO_x and CeO_x:Gd11% were slightly decreased or comparable with the PMA-reference, and there was no observable dose-dependent inhibition of ROS-production. This means that the inhibitory effects on ROS-productions were not stable for CeO_x and CeO_x:Gd11%. The dextran-coated nanoparticles displayed clear inhibition of the ROS-production and the inhibition was related to the amount of cerium present. Dextran-coating provides steric hindrance and can prevent aggregation of nanoparticles, thus preserving the ROS-scavenging properties. In control experiments (no PMA-treatment), the studied nanoparticles did not induce any ROS-production (rather displayed inhibitory effects) at both 15 min and 45 min. Cell viability was evaluated with PrestoBlue following ROS-measurements and there was no significant decrease in viability seen for any sample, verifying the observed inhibitory effects of ROS-production is related to the scavenging properties of the nanoparticles.

Figure 3.7 ROS-production at 15 min and the data is given as percentage of PMA-reference for the studied nanoparticles at [Ce] = 10, 50 and 100 µg/ml. Neutrophils were isolated from whole blood (N=4). ROS production (a.u) ± S.E.M. . * = $P \leq 0.05$ (compared to PMA-reference), □ = $P \leq 0.05$ (compared to Control) Oneway ANOVA (Origin8).

Figure 3.8 ROS-production at 45 min and the data is given as percentage of PMA-reference for the studied nanoparticles at [Ce] = 10, 50 and 100 µg/ml. Neutrophils were isolated from whole blood (N=4). ROS production (a.u) ± S.E.M. * = $P \leq 0.05$ (compared to PMA-reference), □ = $P \leq 0.05$ (compared to Control) Oneway ANOVA (Origin8).

Protein corona formation on $\text{CeO}_x\text{:Gd}$ (DEG) and $\text{CeO}_x\text{:Gd@D/FD}$ were investigated (Paper III and Paper IV) utilizing complementary methods such as Two-dimensional gel electrophoresis (2DGE), MALDI-TOF-MS and nLC-MS/MS. There were numerous of identified proteins bound to the $\text{CeO}_x\text{:Gd}$ nanoparticles, including opsonins. Protein corona covered $\text{CeO}_x\text{:Gd}$ nanoparticles showed cytotoxic properties in a viability assay with monocytes. In contrast to the $\text{CeO}_x\text{:Gd}$ nanoparticles, there were a distinguished reduction of bound proteins on the $\text{CeO}_x\text{:Gd@D/FD}$ nanoparticles as illustrated in Figure 3.9 . In addition, there were no immunoglobulins detected, showing that dextran-coating is a successful approach to increase biocompatibility.

Figure 3.9 Schematic illustration of protein corona formation on above) $\text{CeO}_x\text{:Gd}$ and below) $\text{CeO}_x\text{:Gd@D/FD}$ nanoparticles with time. The figure objects are not in scale.

Key results:

- Gadolinium could be integrated and evenly distributed in the prepared cerium oxide nanoparticles. The integration induced stress in the crystal structure of cerium oxide, thus the size decreased, the shape became elongated and the proportion of Ce^{3+} increased. The nanoparticle diameters were about 5 nm or less for the prepared $\text{CeO}_x\text{:Gd}$ nanoparticles.
- Gadolinium cerium oxide nanoparticles were readily coated with dextran, which enabled dispersibility in cell media. Utilization of FITC-conjugated dextran also enabled fluorescent properties.
- The T_1 -relaxivities of the prepared nanoparticles were dependent on the proportion of gadolinium and highest relaxivity was obtained for $\text{CeO}_x\text{:Gd}<10\%$ nanoparticles, $\sim 12 \text{ mM}^{-1}\text{s}^{-1}$ which is a 3-fold increase compared to the contrast agents in clinic.
- $\text{CeO}_x\text{:Gd}\sim 10\% @ \text{D/FD}$ nanoparticles displayed T_1 -relaxivities above $20 \text{ mM}^{-1}\text{s}^{-1}$, indicating that dextran-coating can increase the T_1 -relaxivity further with approximately a factor of 2.
- The interconversion between Ce^{3+} to Ce^{4+} of $\text{CeO}_x\text{:Gd}$ nanoparticles occurred in presence of ROS (H_2O_2) and by time Ce^{4+} changed back to Ce^{3+} (regenerative interconversion).
- All $\text{CeO}_x\text{:Gd}$ nanoparticles displayed ROS-scavenging properties *in vitro*. The nanoparticle-induced ROS-production was correlating to the gadolinium content of the $\text{CeO}_x\text{:Gd}$ nanoparticles.
- $\text{CeO}_x\text{:Gd}$ nanoparticles show capabilities of ROS-scavenging in whole blood and *in vivo*. In the *in vivo* study, the ROS-scavenging properties were persistent over the entire 5-day period of study.
- $\text{CeO}_x\text{:Gd}$ nanoparticles have an inhibitory effect of platelet activity.
- Dextran-coated $\text{CeO}_x\text{:Gd}$ nanoparticles display stable ROS-scavenging properties over time.
- Dextran-coating reduce the number of proteins bounded to the nanoparticle and prevent adsorption of immunoreactive plasma proteins.

4. Summary of Papers

Paper I

Peter Eriksson, Alexey A. Tal, Andreas Skallberg, Caroline Brommesson, Zhangjun Hu, Robert D. Boyd, Weine Olovsson, Neal Fairley, Igor A. Abrikosov, Xuanjun Zhang & Kajsa Uvdal, *Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement*, **Scientific Reports volume 8, Article number: 6999 (2018)**.

In this paper, cerium oxide nanoparticles were synthesized with integrated Gd-ratios ranging from 0-46%. The prepared samples were characterized by means of crystal structure, colloidal stability, size, shape and relaxivity. CeO_x:Gd9% displayed highest T₁-relaxivity (r_1) 13.4 mM⁻¹s⁻¹, which is about 3 times higher than the most commonly used MRI-contrast agents. CeO_x:Gd nanoparticles with higher Gd-ratios, displayed successively lower T₁-relaxivity. The oxidation states of cerium were investigated with NEXAFS at the synchrotron facility MaxLAB II in Lund. Spectra of Ce³⁺ and Ce⁴⁺ references were measured and the spectral features could be reproduced with theoretical calculations based on density functional theory. The ROS-scavenging properties of the CeO_x:Gd nanoparticles were determined in an *in vitro* model utilizing human neutrophils, PMA and DCF.

Paper II

Peter Eriksson, Anh H.T. Truong, Caroline Brommesson, Ganesh R. Kokil, Robert D. Boyd, Zhangjun Hu, Tram T. Dang, Per Persson, & Kajsa Uvdal, *Cerium Oxide Nanoparticles with Entrapped Gadolinium for High T1 Relaxivity and ROS-scavenging Purposes*, **submitted to ACS Omega**.

In this study, we have synthesized and characterized 5 nm-sized cerium oxide nanoparticles, pure (CeO_x) and with a systematically increased amount of gadolinium (Gd) ion integration, up to 19% (CeO_x:Gd19%). We have shown that by integration of gadolinium ions in cerium oxide nanoparticles, a stable crystalline 5 nm-sized nanoparticulate system with homogenous gadolinium ion distribution is obtained, delivering strong T₁-relaxivity per gadolinium (T₁-relaxivity, r_1 = 12.0 mM⁻¹s⁻¹) and with potential as scavengers of ROS. Specifically, the surface content of Ce³⁺ sites and oxygen vacancies are of utmost importance for antioxidant processes. Characterization of radial distribution of the Ce³⁺, Ce⁴⁺ oxidation states indicated higher concentration of Ce³⁺ at the nanoparticle surfaces. The long-term ROS-scavenging of CeO_x and CeO_x:Gd5% were evaluated in a mice model.

Paper III

Maria Assenhøj, **Peter Eriksson**, Pierre Dönnès, Stefan A. Ljunggren, Kajsa Uvdal, Helén Karlsson & Karin Cederbrant, *Combining Efforts to Evaluate the Toxicity of Metal Oxide Nanoparticles in Human Monocytes and Plasma*, **in manuscript**.

In this study we have investigated a workflow, including characterization of metal oxide NPs and their specific protein coronas followed by assessment of immunotoxic properties *in vitro*. Four different NP types were investigated; Co_3O_4 , Fe_2O_3 , $\text{CeO}_x\text{:Gd}5\%$ and $\text{CeO}_x\text{:Gd}14\%$. Effects on human monocytes *in vitro* as well as potential immunogenicity of proteins in the corona were investigated. A possible adverse outcome pathway upon NP exposure is explored and experimental difficulties are identified and discussed. There were numerous of identified proteins bound to the $\text{CeO}_x\text{:Gd}$ nanoparticles, including immunoglobulins. Protein corona covered $\text{CeO}_x\text{:Gd}$ nanoparticles showed cytotoxic properties in a viability assay with monocytes.

Paper IV

Peter Eriksson, Maria Assenhøj, Caroline Brommesson, Kalle Bunnfors, Reza Nosratabadi, Stefan A. Ljunggren, Helén Karlsson & Kajsa Uvdal. *Gd-CeNPs functionalized with FITC-conjugated dextran for enhanced T1 relaxivity, reduced protein adsorption and ROS scavenging properties*, **in manuscript**.

Here we report successful coating of cerium oxide nanoparticles with integrated gadolinium ($\text{CeO}_x\text{:Gd}$) utilizing dextran conjugated with fluorescein isothiocyanate (FITC). The FITC-conjugated dextran coating enable fluorescent properties, minimizes protein corona formation and improves the dispersion stability in phosphate-based cell media. The prepared dextran-coated $\text{CeO}_x\text{:Gd}$ nanoparticles display an outstanding T1-relaxivity about $20 \text{ mM}^{-1}\text{s}^{-1}$ and have stable over time antioxidant behavior *in vitro*. In contrast to the $\text{CeO}_x\text{:Gd}$ nanoparticles, there was a distinguished reduction of bound proteins on the dextran-coated $\text{CeO}_x\text{:Gd}$ nanoparticles. In addition, there were no immunoglobulins detected, showing that dextran-coating is a successful approach to increase biocompatibility.

Paper V

Peter Eriksson, Caroline Brommesson, Magnus Grenegård & Kajsa Uvdal. *A pilot study on Gadolinium-Integrated Cerium Oxide Nanoparticles in Whole Blood; Platelet Aggregation and Inhibition of ROS-Production*, **in manuscript**.

Here we synthesized and characterized gadolinium-integrated cerium oxide nanoparticles and investigate their interaction with isolated human neutrophils. Nanoparticle neutrophil interaction has been studied utilizing microscopy techniques such as Atomic Force Microscopy, Scanning Electron Microscopy and Transmission Electron Microscopy. In addition the effect of the gadolinium-integrated cerium oxide nanoparticles on primary hemostasis and endogenous ROS generation in whole blood was also evaluated. All synthesized nanoparticle samples exhibited a dose-dependent reduction of both platelet aggregation induced by the collagen-receptor agonist convulxin and scavenging capabilities of reactive oxygen species in whole blood.

Paper VI

Kalle Bunnfors, Natalia Abrikossova, Joni Kilpijärvi, **Peter Eriksson**, Jari Juuti, Niina Halonen, Caroline Brommesson, Anita Lloyd Spets & Kajsa Uvdal, *Nanoparticle activated Neutrophils-on-a chip; A label-free capacitive sensor to measure cells at work*, 2019, **submitted to Sensors and Actuators B**.

In this work, the activity and the response of neutrophil granulocytes are monitored, when externally triggered by exposure of cerium-oxide based nanoparticles, using capacitive sensors based on Lab-on-a-chip technology. This label-free method is used to measure in real-time, oxidative stress of neutrophil granulocytes, minute by minute and visualize the difference in moderate and high cellular work-load during exposure of external triggers

5. Future Perspectives

Recently, U.S. Food and Drug Administration and European Medicine Agency have raised concerns about the MRI contrast agents in clinical use and there is an urgent need for new contrast agents. It is desirable to develop new, safe contrast agents with improved contrast enhancing properties in order to reduce the dose of gadolinium needed. Cerium oxide nanoparticles with gadolinium integrated, have in this thesis, been investigated as promising candidates for the next generation of MRI contrast agents. These type of nanoparticles have two advantages compared to the contrast agents in clinical use today; 1) they contribute to higher MRI signal per gadolinium atom and 2) brings many gadolinium atoms in one agent. Cerium oxide nanoparticles with integrated gadolinium have the potential to provide superior local contrast in MRI. They have also shown the capability to scavenge ROS and thereby they have potential in antioxidant applications. This capability broadens the perspective of gadolinium-cerium oxide nanoparticles. They may in the future be used as a therapeutic agent in oxidative stress related diseases such as Alzheimer's and Parkinson's disease. To summarize, cerium oxide nanoparticles with integrated gadolinium are highly promising for theragnostic purposes within the biomedical field.

The antioxidant and MRI-contrast enhancing capabilities must be further studied in order to fully understand the origin of the theragnostic properties of gadolinium-cerium oxide nanoparticles. These new insights will influence the future nanoparticle design. In synergy with the development of new ROS-probes, more advanced antioxidant assays will give new information about the ROS-scavenging properties of cerium oxide nanoparticles. These assays will be performed not only *in vitro* and *in vivo*, but also in cell free systems where parameters such as concentrations of ROS and nanoparticles are controlled. Investigations of the MRI-contrast enhancing properties are complicated by the numerous parameters that contribute to their relaxivity. Future studies will involve 1) new synthesis strategies where size, shape and composition are further controlled, 2) characterization with high spatial resolution, 3) theoretical modeling and 4) supplementary experiments investigating rotational diffusion and water exchange rates.

The dextran-coating of gadolinium-cerium oxide nanoparticles has been shown to prevent nanoparticle aggregation in cell media, preserve the ROS-scavenging properties and prevent undesirable protein corona formation. The need of an organic coating such as dextran is obvious, but it increases the size (hydrodynamic diameter) of the nanoparticles which may limit the renal excretion. This must be investigated further and the thickness of the organic coating have to be optimized for efficient renal excretion. Further functionalization strategies are planned to enable targeted delivery, which allows the guidance of the theragnostic nanoparticles to a specific site.

6. References

- 1 Feynman, R. P. There's plenty of room at the bottom [data storage]. *Journal of microelectromechanical systems* **1**, 60-66 (1992).
- 2 Organization, W. I. P. World Intellectual Property Report: Breakthrough Innovation and Economic Growth. 10-114 (2015).
- 3 Langer, R. & Tirrell, D. A. Designing materials for biology and medicine. *Nature* **428**, 487 (2004).
- 4 Salata, O. V. Applications of nanoparticles in biology and medicine. *Journal of nanobiotechnology* **2**, 3 (2004).
- 5 Kelkar, S. S. & Reineke, T. M. Theranostics: Combining Imaging and Therapy. *Bioconjugate Chemistry* **22**, 1879-1903, doi:10.1021/bc200151q (2011).
- 6 Xie, J., Lee, S. & Chen, X. Nanoparticle-based theranostic agents. *Advanced drug delivery reviews* **62**, 1064-1079, doi:10.1016/j.addr.2010.07.009 (2010).
- 7 Saraiva, C. *et al.* Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurodegenerative diseases. *Journal of Controlled Release* **235**, 34-47 (2016).
- 8 Choi, H. S. *et al.* Renal clearance of quantum dots. *Nature biotechnology* **25**, 1165 (2007).
- 9 Skotland, T., Iversen, T.-G. & Sandvig, K. New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. *Nanomedicine: Nanotechnology, Biology and Medicine* **6**, 730-737 (2010).
- 10 Hadjidemetriou, M. & Kostarelos, K. Nanomedicine: evolution of the nanoparticle corona. *Nature nanotechnology* **12**, 288 (2017).
- 11 Reddy, L. H., Arias, J. L., Nicolas, J. & Couvreur, P. Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. *Chemical reviews* **112**, 5818-5878 (2012).
- 12 Nel, A. E. *et al.* Understanding biophysicochemical interactions at the nano–bio interface. *Nature materials* **8**, 543 (2009).
- 13 Damhus, T., Hartshorn, R. & Hutton, A. Nomenclature of inorganic chemistry: IUPAC recommendations 2005. *CHEMISTRY International* (2005).
- 14 Hu, B. *et al.* *Handbook of Rare Earth Elements : Analytics*. (De Gruyter, Inc., 2017).
- 15 Henderson, P. *Rare earth element geochemistry*. Vol. 2 (Elsevier, 2013).
- 16 Atwood, D. A. *The rare earth elements: fundamentals and applications*. (John Wiley & Sons, 2013).
- 17 Education, T. J. N. A. F.-O. o. S. *It's Elemental - The Periodic Table of Elements*.
- 18 (NIST), N. I. o. S. a. T. in *NIST Standard Reference Database 111* (2013).
- 19 Trovarelli, A. & Fornasiero, P. *Catalysis by ceria and related materials*. Vol. 12 (World Scientific, 2013).
- 20 Kotani, A., Jo, T. & Parlebas, J. C. Many-body effects in core-level spectroscopy of rare-earth compounds. *Advances in Physics* **37**, 37-85, doi:10.1080/00018738800101359 (1988).

- 21 Schelter, E. J. Cerium under the lens. *Nature Chemistry* **5**, 348, doi:10.1038/nchem.1602 (2013).
- 22 Deshpande, S., Patil, S., Kuchibhatla, S. V. N. T. & Seal, S. Size dependency variation in lattice parameter and valency states in nanocrystalline cerium oxide. *Applied Physics Letters* **87**, 133113, doi:10.1063/1.2061873 (2005).
- 23 Spanier, J. E., Robinson, R. D., Zhang, F., Chan, S.-W. & Herman, I. P. Size-dependent properties of CeO₂-nanoparticles as studied by Raman scattering. *Physical Review B* **64**, doi:10.1103/PhysRevB.64.245407 (2001).
- 24 Celardo, I. *et al.* Ce(3)⁺ ions determine redox-dependent anti-apoptotic effect of cerium oxide nanoparticles. *ACS Nano* **5**, 4537-4549, doi:10.1021/nn200126a (2011).
- 25 Korsvik, C., Patil, S., Seal, S. & Self, W. T. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chemical communications*, 1056-1058 (2007).
- 26 Freeman, B. A. & Crapo, J. D. Biology of disease: free radicals and tissue injury. *Laboratory investigation; a journal of technical methods and pathology* **47**, 412-426 (1982).
- 27 Thannickal, V. J. & Fanburg, B. L. Reactive oxygen species in cell signaling. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **279**, L1005-L1028 (2000).
- 28 Dickinson, B. C. & Chang, C. J. Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nature chemical biology* **7**, 504 (2011).
- 29 Lee, S. S. *et al.* Antioxidant Properties of Cerium Oxide Nanocrystals as a Function of Nanocrystal Diameter and Surface Coating. *ACS Nano* **7**, 9693-9703, doi:10.1021/nn4026806 (2013).
- 30 Szymanski, C. J. *et al.* Shifts in oxidation states of cerium oxide nanoparticles detected inside intact hydrated cells and organelles. *Biomaterials* **62**, 147-154 (2015).
- 31 Das, S. *et al.* Cerium oxide nanoparticles: applications and prospects in nanomedicine. *Nanomedicine* **8**, 26, doi:10.2217/nnm.13.133 (2013).
- 32 Walkey, C. *et al.* Catalytic properties and biomedical applications of cerium oxide nanoparticles. *Environmental Science: Nano* **2**, 33-53 (2015).
- 33 Xu, C. & Qu, X. Cerium oxide nanoparticle: a remarkably versatile rare earth nanomaterial for biological applications. *NPG Asia Materials* **6**, e90, doi:10.1038/am.2013.88 (2014).
- 34 Nicholls, P. Classical catalase: Ancient and modern. *Archives of Biochemistry and Biophysics* **525**, 95-101, doi:https://doi.org/10.1016/j.abb.2012.01.015 (2012).
- 35 Celardo, I., Pedersen, J. Z., Traversa, E. & Ghibelli, L. Pharmacological potential of cerium oxide nanoparticles. *Nanoscale* **3**, 1411-1420, doi:10.1039/C0NR00875C (2011).
- 36 Hollis, F., Kanellopoulos, A. K. & Bagni, C. Mitochondrial dysfunction in Autism Spectrum Disorder: clinical features and perspectives. *Current opinion in neurobiology* **45**, 178-187 (2017).

- 37 Schubert, D., Dargusch, R., Raitano, J. & Chan, S.-W. Cerium and yttrium oxide nanoparticles are neuroprotective. *Biochemical and biophysical research communications* **342**, 86-91 (2006).
- 38 Heckman, K. L. *et al.* Custom Cerium Oxide Nanoparticles Protect against a Free Radical Mediated Autoimmune Degenerative Disease in the Brain. *ACS Nano* **7**, 10582-10596, doi:10.1021/nn403743b (2013).
- 39 Zhou, X., Wong, L. L., Karakoti, A. S., Seal, S. & McGinnis, J. F. Nanoceria inhibit the development and promote the regression of pathologic retinal neovascularization in the Vldlr knockout mouse. *PloS one* **6**, e16733 (2011).
- 40 Park, E.-J., Choi, J., Park, Y.-K. & Park, K. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. *Toxicology* **245**, 90-100 (2008).
- 41 Hussain, S. *et al.* Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. *ACS nano* **6**, 5820-5829 (2012).
- 42 Cheng, G. *et al.* Cerium oxide nanoparticles induce cytotoxicity in human hepatoma SMMC-7721 cells via oxidative stress and the activation of MAPK signaling pathways. *Toxicology in Vitro* **27**, 1082-1088 (2013).
- 43 Lin, W., Huang, Y.-w., Zhou, X.-D. & Ma, Y. Toxicity of cerium oxide nanoparticles in human lung cancer cells. *International journal of toxicology* **25**, 451-457 (2006).
- 44 Pešić, M. *et al.* Anti-cancer effects of cerium oxide nanoparticles and its intracellular redox activity. *Chemico-biological interactions* **232**, 85-93 (2015).
- 45 Perez, J. M., Asati, A., Nath, S. & Kaittanis, C. Synthesis of biocompatible dextran-coated nanoceria with pH-dependent antioxidant properties. *Small* **4**, 552-556, doi:10.1002/smll.200700824 (2008).
- 46 Asati, A., Santra, S., Kaittanis, C. & Perez, J. M. Surface-Charge-Dependent Cell Localization and Cytotoxicity of Cerium Oxide Nanoparticles. *ACS Nano* **4**, 5321-5331, doi:10.1021/nn100816s (2010).
- 47 Alpaslan, E., Yazici, H., Golshan, N. H., Ziemer, K. S. & Webster, T. J. pH-Dependent Activity of Dextran-Coated Cerium Oxide Nanoparticles on Prohibiting Osteosarcoma Cell Proliferation. *ACS Biomaterials Science & Engineering* **1**, 1096-1103, doi:10.1021/acsbiomaterials.5b00194 (2015).
- 48 Wason, M. S. *et al.* Sensitization of pancreatic cancer cells to radiation by cerium oxide nanoparticle-induced ROS production. *Nanomedicine: Nanotechnology, Biology and Medicine* **9**, 558-569 (2013).
- 49 Coey, J. M. D., Skumryev, V. & Gallagher, K. Is gadolinium really ferromagnetic? *Nature* **401**, 35-36, doi:10.1038/43363 (1999).
- 50 Caravan, P., Ellison, J. J., McMurry, T. J. & Lauffer, R. B. Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics and Applications. *Chemical Reviews* **99**, 60 (1999).
- 51 Pyykkö, P. Magically magnetic gadolinium. *Nature Chemistry* **7**, 680, doi:10.1038/nchem.2287 (2015).
- 52 OECD. *Health at a Glance 2017*. (2017).
- 53 AB, N. M. *The Nobel Prize in Physiology or Medicine 2003*. , <<https://www.nobelprize.org/prizes/medicine/2003/summary/>> (2019).
- 54 Bloch, F. Nuclear induction. *Physical review* **70**, 460 (1946).

- 55 Purcell, E. M., Torrey, H. C. & Pound, R. V. Resonance absorption by nuclear magnetic moments in a solid. *Physical review* **69**, 37 (1946).
- 56 AB, N. M. *The Attraction of Spin.*,
<<https://www.nobelprize.org/prizes/physics/1952/speedread>> (2019).
- 57 Weishaupt, D., Köchli, V. D. & Marincek, B. *How does MRI work?: an introduction to the physics and function of magnetic resonance imaging.* (Springer Science & Business Media, 2008).
- 58 Bydder, G., Hajnal, J. & Young, I. MRI: use of the inversion recovery pulse sequence. *Clinical radiology* **53**, 159-176 (1998).
- 59 Hennig, J. Echoes—how to generate, recognize, use or avoid them in MR-imaging sequences. Part II: Echoes in imaging sequences. *Concepts in Magnetic Resonance* **3**, 179-192, doi:10.1002/cmr.1820030402 (1991).
- 60 Ibrahim, M. A. & Dublin, A. B. in *StatPearls [Internet]* (StatPearls Publishing, 2018).
- 61 Lohrke, J. *et al.* 25 years of contrast-enhanced MRI: developments, current challenges and future perspectives. *Advances in therapy* **33**, 1-28 (2016).
- 62 Pierre, V. C., Allen, M. J. & Caravan, P. Contrast agents for MRI: 30+ years and where are we going? *JBIC Journal of Biological Inorganic Chemistry* **19**, 127-131, doi:10.1007/s00775-013-1074-5 (2014).
- 63 Sherry, A. D., Caravan, P. & Lenkinski, R. E. Primer on gadolinium chemistry. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine* **30**, 1240-1248 (2009).
- 64 Girardi, M. *et al.* Nephrogenic systemic fibrosis: Clinicopathological definition and workup recommendations. *Journal of the American Academy of Dermatology* **65**, 1095-1106.e1097, doi:<https://doi.org/10.1016/j.jaad.2010.08.041> (2011).
- 65 Boyd, A. S., Zic, J. A. & Abraham, J. L. Gadolinium deposition in nephrogenic fibrosing dermopathy. *Journal of the American Academy of Dermatology* **56**, 27-30, doi:<https://doi.org/10.1016/j.jaad.2006.10.048> (2007).
- 66 Grobner, T. Gadolinium – a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrology Dialysis Transplantation* **21**, 1104-1108, doi:10.1093/ndt/gfk062 (2006).
- 67 Schieda, N. *et al.* Gadolinium-Based Contrast Agents in Kidney Disease: A Comprehensive Review and Clinical Practice Guideline Issued by the Canadian Association of Radiologists. *Can J Kidney Health Dis* **5**, 2054358118778573-2054358118778573, doi:10.1177/2054358118778573 (2018).
- 68 Clough, T. J., Jiang, L., Wong, K.-L. & Long, N. J. Ligand design strategies to increase stability of gadolinium-based magnetic resonance imaging contrast agents. *Nat Commun* **10**, 1420-1420, doi:10.1038/s41467-019-09342-3 (2019).
- 69 Caravan, P. Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. *Chemical Society Reviews* **35**, 512-523, doi:10.1039/B510982P (2006).
- 70 Lauffer, R. B. Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chemical reviews* **87**, 901-927 (1987).

- 71 Ahrén, M. *et al.* A simple polyol-free synthesis route to Gd₂O₃ nanoparticles for MRI applications: an experimental and theoretical study. *Journal of Nanoparticle Research* **14**, 1-17, doi:10.1007/s11051-012-1006-2 (2012).
- 72 Park, J. Y. *et al.* Paramagnetic Ultrasmall Gadolinium Oxide Nanoparticles as Advanced T1 MRI Contrast Agent: Account for Large Longitudinal Relaxivity, Optimal Particle Diameter, and In Vivo T1 MR Images. *ACS Nano* **3**, 3663-3669, doi:10.1021/nn900761s (2009).
- 73 Boros, E., Gale, E. M. & Caravan, P. MR imaging probes: design and applications. *Dalton Transactions* **44**, 4804-4818, doi:10.1039/C4DT02958E (2015).
- 74 Tan, T. T. Y. *Rare earth nanotechnology*. (Pan Stanford Publishing Pte. Ltd., 2012).
- 75 Berestok, T. *et al.* Tuning branching in ceria nanocrystals. *Chemistry of Materials* **29**, 4418-4424 (2017).
- 76 Pulido-Reyes, G. *et al.* Physicochemical and biological interactions between cerium oxide nanoparticles and a 1, 8-naphthalimide derivative. *Journal of Photochemistry and Photobiology B: Biology* **172**, 61-69 (2017).
- 77 FASS. *Dextran*, <<https://www.fass.se/LIF/result?userType=0&query=dextran>>
- 78 Weissleder, R., Nahrendorf, M. & Pittet, M. J. Imaging macrophages with nanoparticles. *Nature Materials* **13**, 125, doi:10.1038/nmat3780 (2014).
- 79 Gupta, A. K. & Gupta, M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *biomaterials* **26**, 3995-4021 (2005).
- 80 Toth, G. B. *et al.* Current and potential imaging applications of ferumoxytol for magnetic resonance imaging. *Kidney international* **92**, 47-66 (2017).
- 81 Van Hong Nguyen, B.-J. L. Protein corona: a new approach for nanomedicine design. *Int. J. Nanomed.* **12**, 3137 (2017).
- 82 Na, H. B., Song, I. C. & Hyeon, T. Inorganic nanoparticles for MRI contrast agents. *Advanced materials* **21**, 2133-2148 (2009).
- 83 Kang, H. W., Josephson, L., Petrovsky, A., Weissleder, R. & Bogdanov, A. Magnetic resonance imaging of inducible E-selectin expression in human endothelial cell culture. *Bioconjugate chemistry* **13**, 122-127 (2002).
- 84 Bragg William, H. & Bragg William, L. The reflection of X-rays by crystals. *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character* **88**, 428-438, doi:10.1098/rspa.1913.0040 (1913).
- 85 Smart, L. E. & moore, E. A. *Solid state Physics Third Edition*. (2005).
- 86 Speakman, S. A. Estimating crystallite size using XRD. *MIT Center for Materials Science and Engineering*, 03-08 (2014).
- 87 Langford, J. I. & Wilson, A. Scherrer after sixty years: a survey and some new results in the determination of crystallite size. *Journal of applied crystallography* **11**, 102-113 (1978).
- 88 Stetefeld, J., McKenna, S. A. & Patel, T. R. Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophys Rev* **8**, 409-427, doi:10.1007/s12551-016-0218-6 (2016).
- 89 Hallett, F. R. Particle size analysis by dynamic light scattering. *Food research international* **27**, 195-198 (1994).

- 90 Finsy, R. Particle sizing by quasi-elastic light scattering. *Advances in colloid and interface science* **52**, 79-143 (1994).
- 91 Jiang, J., Oberdörster, G. & Biswas, P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. *Journal of Nanoparticle Research* **11**, 77-89 (2009).
- 92 Hunter, R. J. *Zeta potential in colloid science: principles and applications*. Vol. 2 (Academic press, 2013).
- 93 Williams, D. B. & Carter, C. B. *Transmission Electron Microscopy: A Textbook for Materials Science*. (Springer US, 2009).
- 94 Goldstein, J. I. *et al. Scanning electron microscopy and X-ray microanalysis*. 3rd edition edn, (Springer, 2003).
- 95 Haugstad, G. *Atomic force microscopy: understanding basic modes and advanced applications*. (John Wiley & Sons, 2012).
- 96 Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*. Third Edition edn, (Springer, 2006).
- 97 van der Heide, P. *X-ray Photoelectron Spectroscopy : An introduction to Principles and Practices*. (Wiley, 2011).
- 98 Stöhr, J. *NEXAFS spectroscopy*. Vol. 25 (Springer Science & Business Media, 2013).
- 99 Stuart, B. H. *Infrared Spectroscopy : Fundamentals and Applications*. (John Wiley & Sons, Incorporated, 2004).
- 100 Lewis, I. R. & Edwards, H. *Handbook of Raman spectroscopy: from the research laboratory to the process line*. (CRC Press, 2001).
- 101 Gomes, A., Fernandes, E. & Lima, J. L. Fluorescence probes used for detection of reactive oxygen species. *Journal of biochemical and biophysical methods* **65**, 45-80, doi:10.1016/j.jbbm.2005.10.003 (2005).
- 102 Chen, X. *et al.* Recent progress in the development of fluorescent, luminescent and colorimetric probes for detection of reactive oxygen and nitrogen species. *Chemical Society Reviews* **45**, 2976-3016 (2016).
- 103 Jiao, X. *et al.* Small-molecule fluorescent probes for imaging and detection of reactive oxygen, nitrogen, and sulfur species in biological systems. *Analytical chemistry* **90**, 533-555 (2017).
- 104 Chen, X., Zhong, Z., Xu, Z., Chen, L. & Wang, Y. 2', 7'-Dichlorodihydrofluorescein as a fluorescent probe for reactive oxygen species measurement: forty years of application and controversy. *Free radical research* **44**, 587-604 (2010).
- 105 Soehnlein, O., Steffens, S., Hidalgo, A. & Weber, C. Neutrophils as protagonists and targets in chronic inflammation. *Nature Reviews Immunology* **17**, 248, doi:10.1038/nri.2017.10 (2017).
- 106 Liu, W. F. *et al.* Real-time in vivo detection of biomaterial-induced reactive oxygen species. *Biomaterials* **32**, 1796-1801 (2011).
- 107 Nimse, S. B. & Pal, D. Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances* **5**, 27986-28006 (2015).
- 108 Hackel, D. *et al.* The connection of monocytes and reactive oxygen species in pain. *PLoS One* **8**, e63564, doi:10.1371/journal.pone.0063564 (2013).

- 109 Dang, T. T. *Anti-inflammatory drugs for modulation of host response to biomaterials and application in diabetes therapy*, Massachusetts Institute of Technology, (2012).
- 110 Xu, M., McCanna, D. J. & Sivak, J. G. Use of the viability reagent PrestoBlue in comparison with alamarBlue and MTT to assess the viability of human corneal epithelial cells. *Journal of pharmacological and toxicological methods* **71**, 1-7, doi:10.1016/j.vascn.2014.11.003 (2015).
- 111 Jedlinski, A. *et al.* Cetuximab sensitivity of head and neck squamous cell carcinoma xenografts is associated with treatment-induced reduction in EGFR, pEGFR, and pSrc. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* **46**, 717-724, doi:10.1111/jop.12545 (2017).
- 112 Meade, T. *et al.* Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *The Lancet* **328**, 533-537 (1986).
- 113 Hermans, C. *et al.* A compound heterozygous mutation in glycoprotein VI in a patient with a bleeding disorder. *Journal of thrombosis and haemostasis : JTH* **7**, 1356-1363, doi:10.1111/j.1538-7836.2009.03520.x (2009).
- 114 Paniccia, R., Priora, R., Liotta, A. A. & Abbate, R. Platelet function tests: a comparative review. *Vasc Health Risk Manag* **11**, 133-148, doi:10.2147/VHRM.S44469 (2015).
- 115 Wu, W. W., Wang, G., Baek, S. J. & Shen, R.-F. Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel-or LC- MALDI TOF/TOF. *Journal of proteome research* **5**, 651-658 (2006).
- 116 Gustafsson, O. J. *et al.* Matrix-assisted laser desorption/ionization imaging protocol for in situ characterization of tryptic peptide identity and distribution in formalin-fixed tissue. *Rapid Communications in Mass Spectrometry* **27**, 655-670 (2013).
- 117 Lundqvist, M. *et al.* Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proceedings of the National Academy of Sciences* **105**, 14265-14270 (2008).
- 118 Park, D. *et al.* Noninvasive Imaging of Cell Death Using an Hsp90 Ligand. *Journal of the American Chemical Society* **133**, 2832-2835, doi:10.1021/ja110226y (2011).
- 119 Schutte, R. J., Parisi-Amon, A. & Reichert, W. M. Cytokine profiling using monocytes/macrophages cultured on common biomaterials with a range of surface chemistries. *J Biomed Mater Res A* **88**, 128-139, doi:10.1002/jbm.a.31863 (2009).
- 120 Babu, S. *et al.* Dopant-mediated oxygen vacancy tuning in ceria nanoparticles. *Nanotechnology* **20**, 085713, doi:10.1088/0957-4484/20/8/085713 (2009).
- 121 Godinho, M. J. *et al.* Room temperature co-precipitation of nanocrystalline CeO₂ and Ce_{0.8}Gd_{0.2}O_{1.9-δ} powder. *Materials Letters* **61**, 1904-1907, doi:https://doi.org/10.1016/j.matlet.2006.07.152 (2007).
- 122 Eriksson, P. *et al.* Cerium oxide nanoparticles with antioxidant capabilities and gadolinium integration for MRI contrast enhancement. *Scientific Reports* **8**, 6999, doi:10.1038/s41598-018-25390-z (2018).

- 123 Rohrer, M., Bauer, H., Mintorovitch, J., Requardt, M. & Weinmann, H. J. Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. *Investigative radiology* **40**, 715-724 (2005).
- 124 Baldim, V., Bedioui, F., Mignet, N., Margail, I. & Berret, J. F. The enzyme-like catalytic activity of cerium oxide nanoparticles and its dependency on Ce³⁺ surface area concentration. *Nanoscale* **10**, 6971-6980, doi:10.1039/C8NR00325D (2018).
- 125 Abrikossova, N., Skoglund, C., Ahrén, M., Bengtsson, T. & Uvdal, K. Effects of gadolinium oxide nanoparticles on the oxidative burst from human neutrophil granulocytes. *Nanotechnology* **23**, 275101 (2012).

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