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Nur Farhanah Rosli

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**APPLICATIONS OF LAYERED MATERIALS IN
ELECTROCHEMISTRY AND BIOMEDICINE**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2020

APPLICATIONS OF LAYERED MATERIALS IN ELECTROCHEMISTRY AND BIOMEDICINE

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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Authorship Attribution Statement

This thesis contains materials from 5 papers published in the following peer-reviewed journals in which I am listed as an author.

Chapter 2 is published as N. F. Rosli, N. M. Latiff, Z. Sofer, A. C. Fisher, M. Pumera. *Appl. Mater. Today*, **11**, 200-206. (2018)

The contributions of the co-authors are as follows:

- Prof M. Pumera and Prof A. C. Fisher guided through the project directions and vetted the manuscript.
- The manuscript was prepared by N. F. Rosli and revised by Dr N. M. Latiff.
- N. F. Rosli and Dr N. M. Latiff co-designed the studies.
- All the laboratory work, which included materials characterization and cytotoxicity experiments, and analysis of data were performed by N. F. Rosli.
- The materials in the study was synthesized by Prof Z. Sofer.

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The contributions of the co-authors are as follows:

- Prof M. Pumera and Prof A. C. Fisher guided through the project directions and vetted the manuscript.
- The manuscript was prepared by N. F. Rosli and revised by Dr C.C. Mayorga-Martinez and Dr N. M. Latiff.
- N. F. Rosli and Dr C.C. Mayorga-Martinez co-designed the studies.
- Dr N. M. Latiff assisted with cytotoxicity experiments.
- N. Rohaizad assisted with electrochemical experiments
- All the laboratory work, including materials characterization, electrochemical and cytotoxicity experiments, and analysis of data were performed by N. F. Rosli.
- Materials in this study were synthesized by Prof Z. Sofer. He also provided additional materials characterization data such as TEM, SAED, EDS, DLS and XRD.

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- Prof M. Pumera, Prof A. C. Fisher and Prof R. D. Webster guided through the project directions and vetted the manuscript.
- The manuscript was prepared by N. F. Rosli and revised by Dr M. Z. M. Nasir, Dr A. Dash and Prof J. Gonzalez-Julian.
- N. F. Rosli and Dr M. Z. M. Nasir co-designed the studies.
- Dr M. Z. M. Nasir assisted with electrochemical experiments
- All the laboratory work, including materials characterization, electrochemical and cytotoxicity experiments, and analysis of data were performed by N. F. Rosli.
- Prof Z. Sofer and Dr N. Antonatos conducted additional materials characterization and provided relevant data and explanations.
- Dr A. Dash and Prof J. Gonzalez-Julian synthesized the materials in this study.

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- Prof M. Pumera guided through the project directions and vetted the manuscript.
- Prof A. C. Fisher and Prof R. D. Webster provided additional advice and inputs to improve the quality of the project.
- The manuscript was prepared by N. F. Rosli and revised by N. Rohaizad.
- N. F. Rosli and N. Rohaizad co-designed the studies.
- N. Rohaizad assisted with electrochemical experiments
- All the laboratory work, including materials characterization, electrochemical and cytotoxicity experiments, and analysis of data were performed by N. F. Rosli.
- J. Sturala synthesized the materials in the study, conducted additional materials characterization and provided relevant data and explanations.

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- N. F. Rosli and Dr M. Fojtů co-designed the studies.
- All the laboratory work, including materials characterization and cytotoxicity experiments, and analysis of data were performed by N. F. Rosli.

1 August 2020

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Abstract

Layered materials are at the forefront of materials research owing to their exceptional and unique characteristics which varies in both bulk and single-layer forms. Layered materials are versatile materials for a myriad of applications. In this thesis, we extend our studies to recent layered materials including metal carbides and borides, transition metal dichalcogenides, pnictogens and functionalized germanene and silicene. With an increasing demand in clean and renewable energy, layered materials are promising alternatives in electrochemical clean energy applications. As electrochemistry plays an essential role in this field, we aim to expand the current understanding by investigating fundamental electrochemical studies of these layered materials as well as studying them as electrocatalysts for electrochemical sensing and important energy reactions. In addition, their toxicological properties need to be well understood in order to recognize any safety hazards that they may pose to humans and the environment. Hence, we supplement our investigations with safety studies on these materials to allow a preliminary gauge on their toxic impacts. Lastly, we expand our research on layered materials to biomedicine, specifically as anticancer drug carriers with high loading capacity due to their nanoscale and two-dimensional nature. These studies are vital in contributing to the pool of ongoing research on layered materials and their progress towards commercialization in electrochemical and biomedical applications.

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List of Publications

1. **Nur Farhanah Rosli**, Carmen C. Mayorga-Martinez, Adrian C. Fisher, Osamah Alduhaish, Richard D. Webster, Martin Pumera. *Arsenene nanomotors as anticancer drug carrier*. ***Appl. Mater. Today***, **2020**, *21*, 100819.
2. **Nur Farhanah Rosli**, Nasuha Rohaizad, Jiri Sturala, Adrian C. Fisher, Richard D. Webster, and Martin Pumera. *Siloxene, Germanane, and Methylgermanane: Functionalized 2D Materials of Group 14 for Electrochemical Applications*. ***Adv. Funct. Mater.*** **2020**, *30*, 1910186.
3. **Nur Farhanah Rosli**, Muhammad Zafir Mohamad Nasir, Nikolas Antonatos, Zdeněk Sofer, Apurv Dash, Jesus Gonzalez-Julian, Adrian C. Fisher, Richard D. Webster, Martin Pumera. *MAX and MAB Phases: Two-Dimensional Layered Carbide and Boride Nanomaterials for Electrochemical Applications*. ***ACS Appl. Nano Mater.***, **2019**, *2*, 6010-6021.
4. **Nur Farhanah Rosli**, Michaela Fojtů, Adrian C. Fisher, Martin Pumera. *Graphene Oxide Nanoplatelets Potentiate Anticancer Effect of Cisplatin in Human Lung Cancer Cells*. ***Langmuir***, **2019**, *35*, 3176-3182.
5. **Nur Farhanah Rosli**, Carmen C. Mayorga-Martinez, Naziah M. Latiff, Nasuha Rohaizad, Zdeněk Sofer, Adrian C. Fisher, Martin Pumera. *Layered PtTe₂ Matches Electrocatalytic Performance of Pt/C for Oxygen Reduction Reaction with Significantly Lower Toxicity*. ***ACS Sustain. Chem. Eng.***, **2018**, *6*, 7432-7441.
6. **Nur Farhanah Rosli**, Naziah M. Latiff, Zdeněk Sofer, Adrian C. Fisher, Martin Pumera. *In Vitro Cytotoxicity of Covalently Protected Layered Molybdenum Disulphide*. ***Appl. Mater. Today***, **2018**, *11*, 200-206.
7. Tijana Maric, Muhammad Zafir Mohamad Nasir, **Nur Farhanah Rosli**, Maja Budanović, Richard D. Webster, Nam-Joon Cho, Martin Pumera. *Microrobots Derived from Variety Plant Pollen Grains for Efficient Environmental Clean Up and as an Anti-Cancer Drug Carrier*. ***Adv. Funct. Mater.*** **2020**.
8. Naziah M. Latiff, **Nur Farhanah Rosli**, Carmen C. Mayorga-Martinez, Katerina Szokolava, Zdeněk Sofer, Adrian C. Fisher, Martin Pumera. *MnPS₃ shows Anticancer Behavior Towards Lung Cancer Cells*. ***FlatChem***, **2019**, *18*, 100134.
9. Lei Kong, **Nur Farhanah Rosli**, Hui Ling Chia, Jianguo Guan, Martin Pumera. *Self-Propelled Autonomous Mg/Pt Janus Micromotor Interaction with Human Cells*. ***B. Chem. Soc. Jpn.***, **2019**, *92*, 1754-1758.
10. Tijana Maric, Muhammad Zafir Mohamad Nasir, Carmen C Mayorga-Martinez, **Nur Farhanah Rosli**, Maja Budanović, Kateřina Szököllová, Richard D Webster, Zdenek Sofer, Martin Pumera. *Cloisite Microrobots as Self-Propelling Cleaners for Fast and Efficient Removal of Improvised Organophosphate Nerve Agents*. ***ACS Appl. Mater. Interfaces.*** **2019**, *11*, 31832-31843.

11. Qi Dong, Naziah M. Latiff, Vlastimil Mazánek, **Nur Farhanah Rosli**, Hui Ling Chia, Zdeněk Sofer, Martin Pumera. *Triazine-and Heptazine-based Carbon Nitrides: Toxicity.* ***ACS Appl. Nano Mater*, 2018, 1, 4442-4449.**

Objectives of Thesis

The thesis titled “*Applications of Layered Materials in Electrochemistry and Biomedicine*” has an overall objective of exploring recent layered materials for various electrochemical energy and drug delivery applications. Non-renewable fossil fuels still remain as one of our primary sources of energy but they lead to several negative consequences as they generate an enormous carbon footprint. Additionally, the supply for non-renewable fossil fuels are currently depleting due to an exponential increase in energy demand caused by advanced living standards and higher number of human activities. As the society moves towards more sustainable and cleaner forms of energy, electrochemical energy applications hold a very crucial role. Particularly, recent studies mainly focus on hydrogen-based fuel cells which use solely hydrogen and oxygen gas to produce energy, releasing water as the only product. Layered materials have been proven to be promising alternative catalysts for these cleaner energy generation processes in hydrogen-based fuel cells. Utilising materials in drug delivery applications mainly aim to improve pharmacokinetics and biodistribution of drugs or/and to function as drug reservoirs. Generally, drug delivery systems should result in enhancement of several crucial properties of “free drugs” such as, improving solubility, in vivo stability and specificity, reducing side effects and tissue damage as well as enhancing drug efficacy. Layered materials have been proven to have large surface-to-volume ratio, especially when the layers are separated into two-dimensional nanosheets. This is an ideal characteristic for drug delivery systems as it provides more binding sites and hence, possess higher loading capacity of both drugs and other molecules for enhancement of other properties. This thesis focuses on two main parts; the potential electrochemical and drug delivery applications of layered materials.

Before embarking on the respective application studies, it is important to understand their background and the currently reported studies. We begin with an introduction and literature

reviews in three important sections in **Chapter 1**. The first section will give a general background on layered materials and safety studies that are currently reported by the scientific community. Following that, the second section will discuss briefly on fundamental electrochemistry and currently reported layered materials for electrochemical energy applications. The third and last section of this chapter will provide a brief background on layered materials in biomedicine with a literature review focusing on layered materials in drug delivery.

Part I of this thesis is titled “*Electrochemical Applications and Safety Studies of Layered Materials*”. There are total of four projects presented in this part. We start by exploring cytotoxicity of a covalently protected molybdenum dichalcogenide in **Chapter 2**. Following that, the electrochemical applications and cytotoxicity studies of three different groups of layered materials are explored in **Chapter 3** (layered platinum dichalcogenides), **Chapter 4** (layered carbide and boride: MAX and MAB phases) and **Chapter 5** (functionalized group 14 layered materials: siloxene, germanene and methylgermanene) respectively.

Thereafter, **Part II** is titled “*Layered Materials in Biomedicine*”. This chapter explores the capabilities of layered graphene oxide (**Chapter 6**) and layered arsenene (**Chapter 7**) as drug carriers in drug delivery applications for cancer therapy.

Lastly, **Chapter 8** concludes this thesis with a short summary as well as future directions to embark towards in this field of study.

Chapter 1

Introduction and Literature Review

1.1 Layered Materials

1.1.1 A Background

Layered materials have several unique and promising properties mainly due to their anisotropy. Taking example from graphite, these stacked graphene monolayers were shown to vary in electrical and thermal conductivities along the basal plane and than the edge plane. In addition, intensive research on graphene and its derivatives demonstrates that nanoscale confinement could alter the properties of graphite upon exfoliation to monolayer or few layers.

The isolation of one-layer graphene by a simple “scotch tape” mechanical cleavage method from graphite has since sparked interests in layered materials.¹ Graphite is made up of several graphene layers that are weakly bonded by van der Waals interactions. These individual graphene sheets can be easily obtained by a variety of techniques, mainly mechanical, chemical and electrochemical exfoliation from the bulk graphite.^{2,3} The two-dimensional carbon atoms in graphene possessed a honeycomb lattice structure connected by strong sp^2 covalent bonds.⁴ Its freestanding, two-dimensional nature is the reason for its remarkable and unique characteristics that are absent in its bulk counterpart, graphite.

Graphene possess high mechanical strength, flexibility, high thermal and electrical conductivity, large specific surface area and low manufacturing cost.⁵ More importantly, single-layer graphene exhibit properties consistent with a zero band gap semiconductor with massless Dirac particle feature as well as high intrinsic carrier mobility.^{1,6} Interestingly, the oxidation of graphene to form graphene oxide results in improvements to solubility, dispersibility and biocompatibility.⁷ Preparation of graphene oxide is commonly achieved through oxidation with nitric acid and sulfuric acid in strong oxidizing agents.⁸ There are currently several commonly known oxidation methods for graphite, namely; Staudenmaier,

Hoffman and Hummers' oxidation methods.⁹⁻¹¹ These chemical means methods are often preferred due to ease of large scale production.

However, the limitation of electronic structure in graphene has motivated the pursuit of other layered materials as alternative and versatile materials for applications such as catalysis, sensing, electronic, energy storage and biomedicine. With their stacked sheets arrangement, layered materials present unique opportunities for tuning of size and volume with high surface area and controlled modification of sites and properties.¹² To date, the reported layered materials can be classified into mono-, di- and tri-elemental layered materials.¹³ This thesis aims to fill the research gap in current literature by exploring unreported electrochemical and biomedical applications of layered materials and their novel modifications. The studies herein, focus on materials which have yet to be investigated for electrochemical energy and drug delivery applications. The chosen materials consist of monoelemental materials from group 14 (siloxene, germanene and methylgermanene) and group 15 (arsenene), dielemental materials from group 6 (MoS_2) and group 10 (platinum dichalcogenides) as well as trielemental materials MAX and MAB phases. The background of the respective materials will be elaborated further in the following paragraphs.

From group 14, silicene and germanene have been reported as monoelemental layered materials. Silicene and germanene, also known as graphene analogues, are single-element 2D-Xene due to the formation of sp^2 -hybridized alkene bond.^{14,15} Their atoms are generally arranged in a honeycomb lattice structure.¹⁶ These germanium and silicon analogues of graphene were initially studied in 1994 by Takeda and Shiraishi.¹⁷ Silicene was first given its name by Guzman-Verri et al. in 2007.¹⁸ Unlike planar graphene, they uniquely possess varying degrees of buckling which are a result of their larger interatomic distances.¹⁴ This buckling

effect allows more opportunities for covalent functionalization of the layers forming 2D-Xenes. The first few syntheses of silicene was reported on substrates and does not exist as freestanding sheets.¹⁹ The inherent thermodynamic preference for sp^3 bonding is the reason for the low stability of Xenes under ambient conditions.^{14,20}

Covalent functionalization on Xenes is one method of tuning their properties to achieve improved performance and stability. Tuning of their electronic properties can be attained with different substrates, functionalization and strain and this is necessary for subsequent applications in nanoelectronics.^{14,21} Synthesis of functionalized Xenes, also known as Xanes, have been uniquely derived by topotactic exfoliation of their Zintl phases.^{14,22} Zintl phases $CaGe_2$ and $CaSi_2$ have crystal structures composing of anionic $[Si]_n^-$ and $[Ge]_n^-$ layers with similar puckered honeycomb structure to silicene and germanene but has divalent cations between each layer. These Zintl phases can react with electrophilic species forming multi-layered Xanes with the divalent cation solubilized by a solvent.²²⁻²⁴ The first synthesis of hydrogen-terminated germanene, also known as germanane, was obtained by topochemical deintercalation of its layered intermetallic Zintl phase precursor, $CaGe_2$.^{22,23} Meanwhile, the topotactic deintercalation of $CaSi_2$ forming functionalized silicene dates back to 1860s by Wöhler and Kautsky in the 1920s.^{25,26}

Other commonly reported monoelemental layered materials are from group 15 elements, also known as pnictogens.^{27,28} The research on pnictogens started as early as 1914 with studies on phosphorus.²⁹ Rhombohedral arrangement (**Figure 1**) is most stable arrangement for bulk heavy pnictogens (As, Sb and Bi), whereby each layer are stacked together with strong forces of interactions.³⁰ Weak van der Waals forces are not formed as there are differences in the atomic orbital interaction between individual double layers.³⁰ Shortly after the synthesis of

ultrathin 2D black phosphorus as field effect transistors,³¹ the focus channelled towards 2D monolayers of other elements in the same group specifically; arsenene, antimonene and bismuthene.³²⁻³⁶ Their monolayer arrangements can exist in a buckled honeycomb, square-octagon and washboard structures. They are also semiconductors with bandgaps favourable for various device applications.^{28,37}

Figure 1. Rhombohedral structure of heavy pnictogens. Adapted from reference 30.

Transition metal dichalcogenides (TMDs) are a popular dielemental layered materials (**Figure 2a**) represented by the general formula MX_2 (M= transition metal, X= chalcogen).^{38,39} TMDs form graphite-like layered structures with strong anisotropy in their mechanical, thermal and chemical properties. Typically, each layer is 6 to 7 Å thick with two chalcogen atoms layers sandwiching one layer of hexagonal arranged metal atoms.³⁸ The intralayer M-X interactions are bonded covalently while the interlayers are connected with weaker van der Waals interactions, therefore allowing easy cleaving of layers.^{38,39} There exist three common types of polymorphs in bulk TMDs namely; 1T, 2H and 3R (T= trigonal, H= hexagonal and R= rhombohedral).^{38,39} Different polymorphs have been reported to exhibit varying properties and performances.^{40,41} Bulk TMDs have diverse electronic properties varying from insulators to

semiconductors, semimetals and metals.^{38,42} Exfoliation to mono- or few- layers is one method to alter these properties due to quantum confinement effects.^{42,43} Monolayer TMDs exhibit two types of metal coordination namely; trigonal prismatic or octahedral phase (**Figure 2b and 2c**).^{38,39,42} Depending on the transition metal and chalcogen combinations, one coordination mode is usually thermodynamically preferred.

Group 6 TMDs, specifically MoX_2 and WX_2 , have been most widely studied among the various TMDs.⁴⁴⁻⁴⁷ TMDs of group 4 and 5 transition metals are also rapidly gaining interest in recent studies,⁴⁸⁻⁵² while few studies have reported on other TMDs from group 7, 8, 9 and 10 transition metal.⁵³⁻⁵⁸ TMDs possess unique characteristics including a direct bandgap, atomic-scale thickness and superior mechanical and electronic properties.^{39,59} These properties are favourable for electronics, catalysis, energy and medicine.^{39,59} Tuning electronic properties, solubility, stability, catalytic performance, and biocompatibility can be achieved through functionalization of TMDs.⁵⁹⁻⁶² MoS_2 remains to be the most commonly reported functionalized TMD with successfully enhanced properties.^{60,62-66} Specifically, functionalization of MoS_2 has shown changes in solubility, electronic, optoelectronic and magnetic properties as well as possess promising potential for gas sensing, DNA detection, biomedicine and electrochemistry.^{60-62,65,67-69}

Figure 2 a) Different combinations of layered TMDs highlighted in the periodic table, **b)** trigonal prismatic (D_{3h}) and **c)** octahedral (O_h) coordination where the purple and yellow atoms represents a transition metal and chalcogen, respectively. AbA and AbC represents the stacking arrangements in which chalcogen atom is represented by upper-case letters represent and transition metal atom is represented by lower-case letters. Adapted from reference 38.

A tri-elemental layered material of interest is MAX and MAB phases or metallic ceramics.⁷⁰ MAX phases are specifically represented by M_{n+1}AX_n (M = metal, A = IIIA or IVA group element, X = C or N and n = 1-3).^{71,72} These layered transition metal carbides and nitrides have

hexagonal crystal structures in $P6_3/mmc$ symmetry. The A atoms are sandwiched between M and X layers with strong interlayer bonds.⁷² MAB phases are layered transition metal borides and are represented by $(MB)_{2n}A_m(MB_2)_x$ ($n = 1-2$; $m = 1-2$; $x = 0-2$).^{73,74} The properties of MAX and MAB phases are a combination of a metal and ceramics with favourable electrical conductivity, machinability, resistant to thermal shock and damage, possess high temperature strength and elastic moduli as well as resistant to oxidation and corrosion.^{70-72,74} MAX phases can form M-X monolayer structures, MXene, by undergoing selective etching and exfoliation.^{75,76} MAB phases, on the other hand, exhibit orthorhombic crystal structures with different space groups such as $Cmcm$ ($MAIB$), $Cmmm$ (M_2AlB_2 & M_4AlB_6), and $Pmmm$ (M_3AlB_4).^{73,74} Selective etching and exfoliation of MAB phases can similarly produce two-dimensional M-B layers known as MBene.⁷⁷⁻⁷⁹

1.1.2 Safety Studies

With the fast advancements in nanotechnology, many novel materials have been extensively studied and layered materials show several promising potentials in several applications. Layered materials are currently researched for applications in energy, environment, biomedicine, electronics, sensing and many more.⁸⁰⁻⁸³ As of March 2015, a total of 1814 nanomaterial-containing consumer products have been listed. It is also expected that by 2024, the global nanotechnology industry will likely exceed US\$ 125 billion. An estimate of 66000 metric tons per year of nanomaterials are released into surface water globally. In addition, modifications to layered materials, such as functionalization, doping, etching and exfoliation for improved properties and performances can drastically alter their toxicity.^{82,84-86} Hence, toxicity of these novel materials should be carefully studied in its utilized form, prior to any large scale advancements into the respective industries as they may pose possible health hazards to humans and the environment.⁸⁷⁻⁹¹

In the past, we have observed nanomaterials negatively affecting human health when they are commercialized without fully understanding toxic properties. For instance, there was an incident involving Magic-Nano products in 2006, specifically, aerosols designed to coat glass and ceramic.⁹² Due to inhalation of toxic nanoparticles from the products, about 100 consumers became ill and a small number were hospitalized within days. They developed several symptoms such as coughing, headaches, sleep disruption, vomiting and a handful was even diagnosed with pulmonary oedema. This shows how nanomaterials can greatly affect the respiratory system due to its nano-sized characteristic and ease of transmission through inhalation. Also, the US Food and Drug Administration found carbohydrate-coated superparamagnetic iron oxide nanoparticles, ferumoxytol, for anemia treatment to induce lethal anaphylactic reactions. The European Union categorize TiO₂ as a category 2 carcinogen due to

its inhalation hazard and from the start of 2020, France banned use of TiO₂ (E171) in food because of its carcinogenic potential. Therefore, it is of great importance to characteristically, meticulously and accurately study and understand the risks of novel materials to humans and the environment prior to any large scale utilisation.⁹³

Nanomaterials are materials with two or three of its dimensions with lengths ranging from 1 to 100 nanometres. There is a potential for nanomaterials exposure throughout the product chain, specifically during manufacture, application and waste management. Nanomaterials can be easily aerosolized and transmitted to the air during synthesis, handling and processing in laboratory and manufacture settings. Due to this, inhalation of toxic nanoparticles is the primary route of exposure into a human body.⁹⁴ Thus far, many toxicity studies of emerging nanomaterials are conducted on the respiratory system.⁹⁴ Nevertheless, there are other routes of exposure which includes skin, liver, blood brain barrier, gastrointestinal tract and spleen which are vital organs in a human body.⁹⁵ This can be administered through vast applications of nanomaterials in biomedicine which is exceedingly increasing.⁹⁶ In general, layered nanomaterials are likely to have adverse effects at their portal of entry but may eventually translocate to other organs in the body. With more thorough assessments addressing the risks and safety of nanomaterials, the possible dangers and threats which nanomaterial may pose can be well comprehended, and proper regulations can be set to protect humans and the environment.⁹³

Generally, particles are harmful as a consequence of two factors that act together; large surface area and intrinsic toxicity of the surface. Hence, nanomaterials are highly harmful due to their nano-size nature as smaller particles have more surface area per unit mass. The toxicity of nanomaterials is highly influenced by their interactions with biological systems, which can in

turn be affected by the physicochemical properties, such as shape, size, chemical composition, surface coating and charge as well as agglomeration or aggregation tendency.^{91,97} Therefore, thorough characterization of materials is necessary so as to determine an accurate correlation between these physicochemical properties and its toxicological effects.⁹⁸ *In vitro* assessments can determine not only cell death due to a potentially toxic agents, but also other changes in cellular functions due to the materials. Changes can be observed to the cell's metabolic activity, membrane integrity, cell proliferation capabilities and signs of apoptosis.⁹¹ *In vitro* assessments are more economical, fast and avoid ethical issues from animal studies unlike *in vivo* studies, which makes it an ideal method for immediate and preliminary investigations. Generally, *in vitro* assessments involve cell-culture studies after exposing them to materials for a designated period.

Due to varying effects of toxic materials on the cells, there are many types of *in vitro* studies which are aimed to investigate different changes to the cellular functions. Cell viability studies are those which mainly investigate changes in the metabolic activity of cells.⁹⁹ Two simple and commonly used assays which measure cell viabilities are 1) methylthiazolyldiphenyl-tetrazolium bromide (MTT) and 2) water-soluble tetrazolium salt (WST-8) assays. These are well-established colorimetric-based tetrazolium reduction assays for high throughput screenings. They have similar fundamentals whereby tetrazolium reduces to formazan, in the presence of metabolic activity in cells, and subsequently generate dyed products for detection and quantification.¹⁰⁰

There is simpler toxicity test which require mere visual inspection of cells for changes in cellular and nuclear morphology with bright-field microscopy but overall, colorimetric-based methods are more common. Colorimetric-based methods can be categorized into measuring of

either 1) plasma membrane integrity and 2) mitochondrial activity.⁸⁸ For instance, a weak cationic dye, known as neutral red assay, can cross the plasma membrane of a live cell by diffusion, allowing clear distinguishment between live and dead cells and quantifiable through spectroscopic measurements.¹⁰¹ LIVE/DEAD viability test is similar as it utilises calcein acetoxymethyl (calcein AM) and ethidium homodimer dyes for easy labelling of live and dead cells. Calcein AM can easily diffuse into the cells and subsequently converted to green fluorescent molecules in the presence of intracellular esterase of live cells. Ethidium homodimer is a red fluorescent molecule when binded to the nucleic acids of dead cells. The live and dead cells can be quantified by fluorescence microscopy at wavelength of 515 nm (calcein AM) and 635 nm (ethidium homodimer), respectively, when excited at 495 nm.¹⁰²

The chosen assays for layered materials safety studies in this thesis are MTT and WST-8 cell viability assays, which measure mitochondrial activity, as well as LIVE/DEAD viability test, which measures plasma membrane integrity.

1.2 Electrochemical Applications of Layered Materials

1.2.1 Fundamental Electrochemistry of Layered Materials

The downsizing of graphite to graphene sheets have been known to unlock a unique set of properties, specially for electrochemical applications.^{2,103,104} The two-dimensional environment of graphene is ideal for electron transport and heterogeneous electron transfer (HET) kinetics, exemplifying exceptional electronic conductivity.¹⁰⁵ HET was found to take place only on the edges while the basal plane remains inert.¹⁰⁶ Additionally, Papakonstantinou et al. found that high amounts of edge sites on synthesized graphene nanoplatelets are the

reason for fast HET kinetics and sensitive detection of dopamine.¹⁰⁷ The abundance of edge planes in stacked graphene sheets is crucial for fast electron transfer kinetics, electrocatalytic and biosensing properties.⁴ This anisotropic electronic property of stacked graphene sheets propelled the research on electrochemical studies of materials with abundant edge sites; layered materials.

The heterogeneous electron transfer (HET) is a fundamental process in an electrochemical reaction whereby electrons transfer between electrode materials and electroactive molecular probes in the solution.¹⁰⁸ Investigating the HET rate of a material allows deeper understanding of electrode kinetics and mechanism involved.^{109,110} Fast HET rate would indicate higher efficiency in electron transfer between the solid-state substance on an electrode and electroactive probes in solution, suggesting suitability of material for applications such as sensing. Ideally, materials used for sensing should possess large surface area and abundant sites for efficient and selective interaction of probes.¹¹¹ Layered materials are ideal for sensing applications as they exhibit large surface ratio, possess rich surface chemistry for efficient interaction with targeted species and have been reported with fast HET rates.^{112,113} For instance, the TMD, MoS₂ nanoparticles, were reported to successfully electrochemically sense very low amounts H₂O₂ from living cells.¹¹⁴ To date, several studies have shown layered materials-based sensors with promising potential for field effect transistor (FET)-based sensor, biosensing, pesticide detection and gas sensing.¹¹⁵⁻¹¹⁸

Structural and chemical modifications may occur during electrochemical use of layered materials on an electrode surface and this may directly affect the efficiency and reliability of the materials as electrochemical devices. Inherent properties of the material can be one of the

reasons for material instability but it is also dependent on other experimental conditions such as electrolyte, applied potential window and pH.¹⁰⁸ Inherent electrochemistry is the intrinsic oxidation and reduction of a material when an electrochemical potential is applied.¹⁰⁹ For instance, pristine graphene shows negligible inherent electrochemistry but chemically modified graphene, which possess oxygen modifications, can undergo electrochemical reductions mainly due to electroactive oxygen functionalities.^{119,120} Transition metal dichalcogenides (TMDs) also possess inherent properties as the transition metal readily undergoes oxidation.^{121,122} Nevertheless, the chalcogen was proven to affect the intrinsic oxidation potential of the TMDs.^{52,56} Hence, prior identification of inherent redox properties is essential as it can greatly affect electrochemical potential of layered materials by limiting the operating potential window of materials or contributing to interferences in peak observations in an electrochemical scan.^{123,124}

1.2.2 Electrochemical Energy Applications

Over the years, our demand for energy has increased due to advanced living standards as well as higher number of commercial and industrial activities. We are now facing a global energy crisis as resources for energy production are drastically depleting and the current methods of energy generation are heavily contributing to the problem of climate change.^{125,126} Non-renewable energy remains to be the primary source of energy but the search for more efficient, sustainable and cleaner energy alternatives are actively is pursuit.¹²⁷ One promising field of research which is directed to this purpose is clean energy generation technologies. In recent research, more focus has been devoted to energy conversion processes related to hydrogen production as clean energy fuel.^{128,129} Particularly, hydrogen-based fuel cells use solely hydrogen and oxygen gas to produce energy, releasing water as the only product. Electrocatalysis is a key part of these clean energy conversion in future technologies.^{130,131}

In hydrogen fuel cells, some important reactions are hydrogen evolution (HER), hydrogen oxidation (HOR), oxygen evolution (OER) and oxygen reduction (ORR) reactions. Water electrolysis process involves OER and HER at the anode and cathode, respectively, in an electrolytic cell forming hydrogen gas as fuel and oxygen gas as by-products. The reverse reaction involves HOR and ORR at the anode and cathode, respectively, from the hydrogen fuel gas and oxygen gas to produce renewable energy with water as a clean by-product.^{132,133} However, these reactions require catalysts for to be carried out efficiently. Expensive and rare platinum-based catalysts, IrO_2 and RuO_2 remain as the best materials for these reactions.¹³⁴⁻¹³⁶ Hence, there are various ongoing research aimed to uncover active, sustainable and more abundant materials as catalysts.

In recent years, layered materials were found to possess promising properties as economical alternatives for catalysts. Layered transition metal hydroxides and oxides were reported with favourable properties for ORR and OER.¹³⁷⁻¹³⁹ It was found that their catalytic activity is largely related to oxygen vacancies and formation of sub-oxides on these materials.¹³⁷ In addition, they are commonly utilized in a hybrid structure with carbon materials that has high surface area, such as graphene, as they were proven to synergistically result in promising catalytic properties.¹⁴⁰⁻¹⁴² Graphene hybrids, which consist of three-dimensional graphene framework and two-dimensional materials, have been broadly studied and were shown to possess superior performances and promising properties for various energy applications.¹⁴³

Transition metal dichalcogenides (TMDs) are layered materials which have proven their superiority for HER.^{108,109} Edges sites of TMDs are found to be most catalytically active for HER electrocatalysis. This was experimentally shown by MoS₂ catalyst in its 2H phase.¹⁴⁴ In **Figure 3**, the exchange current density (i_0) was plotted as a function of the free energy of adsorption of hydrogen (ΔG_{H^*}), which was calculated by density functional theory (DFT).^{144,145} A volcano plot was observed for MoS₂ with other pure metals suggest that the HER catalytic performance of MoS₂ at its edge site is almost as ideal as platinum (Pt) catalyst, which require negligible overpotential to achieve superior HER performance.¹⁴⁶ As a consequence, other group 6 TMDs were explored as catalyst for HER, specifically MoSe₂, WS₂ and WSe₂.^{122,147,148} Other TMDs from other groups were then studied for their HER catalytic activity and their performances are summarized by Lin et al.¹⁴⁹

Figure 3. “Volcano” plot of current density (i_0) vs Gibbs free energy of adsorbed atomic hydrogen (ΔG_{H^*}) for MoS₂ and other pure metals. Republished from references 144 - 146.

Metal-free catalysts, such as the carbon-based ones, are more favourable as the non-metal elements generally have higher abundance, lower toxic effects on the environment and are more economical. Although pristine carbon is ineffective for ORR, OER and HER electrocatalysis, a few performance improving strategies such as chemical doping, structural defects and physical intermolecular charge transfer have been employed.¹⁵⁰⁻¹⁵³ For ORR, carbon-based catalysts can undergo strategies such as controlled porosity, regulated doping/co-doping configurations and building structural microarchitectures for sp² carbon for improved catalysis performance.¹⁵⁰ Li et al. synthesized highly abundant micropores and edge defects on nitrogen-doped carbon nanosheets to achieve high specific surface area and abundant active sites for ORR, OER and HER electrocatalysis.¹⁵¹ Besides that, layered pnictogens, such as black phosphorus, are another non-carbon metal-free alternatives explored as electrocatalysts for various energy applications.¹⁵⁴⁻¹⁵⁷

1.3 Layered Materials in Biomedicine

1.3.1 A Brief History

The two-dimensional material, graphene, presents novel opportunities for biomedicine due to its unique attributes such as nano-scale size with large surface area for high loading capacity, biofunctionalization capabilities for improved biocompatibility and favourable mechanical, electronic and optical properties for bioimaging.¹⁵⁸⁻¹⁶⁰ Additionally, graphene and its derivatives can form hydrophobic interactions and supramolecular π - π stacking easily with specific compounds as a result of their large surface area and sp^2 -bonded carbonaceous surface. To date, graphene has proven its performance for several biomedical applications such as genetic and tissue engineering, bioimaging, biosensing, immunosensing, cancer phototherapy and drug delivery.¹⁵⁸

The concept of obtaining nanosheets from its bulk, layered counterparts has extended to other layered materials with similar promising physical and chemical properties for biomedicine. Layered double hydroxides, transition metal oxides and hydroxides, pnictogens, transition metal dichalcogenides (TMD) and MXenes are layered materials that are also regarded as graphene analogues. Over the years, graphene and its analogues have presented favourable potentials for several biomedical diagnostics and therapeutic applications such as biosensing, immunosensing, bioimaging, genetic and tissue engineering, drug delivery and cancer therapy.^{82,83,85,158-163} For instance, TMD nanosheets have been used as photothermal platforms for cancer treatment due to high absorbance in NIR range. MoS_2 and WS_2 were reported as NIR absorbing agent and photosensitizer carrier for photothermal and photodynamic therapies.^{68,164-166} Uniquely, TMD possess thickness-dependent optical and electrical properties, which when combined with their large surface area for drug loading, result in promising drug delivery

carriers for cancer therapy. Another example is black phosphorus, in which their few-layered form were proven to degrade quickly under ambient conditions to phosphate anions, yet is a favourable property for biomedical applications as it results in biocompatibility and biodegradability.¹⁶⁷ This concludes that thickness of the layers play a crucial role in determining the property of the material and can define their applicability in biomedical applications.

1.3.2 Layered Materials in Drug Delivery

Specifically, in cancer treatment, layered materials have demonstrated promising properties for photothermal and photodynamic therapy, chemotherapy as well as drug loading and delivery. One of the outstanding characteristics of layered material, especially when downsized to two-dimensional nanosheets, is its large surface-to-volume ratio. This structural property is highly useful for drug loading as it provides numerous anchor points for binding of other molecules. This allows high loading capacity of anticancer drugs which is an essential characteristic for materials chosen for drug delivery.

In more recent literature reports, a diverse array of layered materials have been highlighted as novel materials for drug delivery applications. For instance, monoelemental materials such as Xenes (borophene, silicene, germanene, phosphorene, stanene, antimonene, bismuthene, arsenene, tellurene) were utilized in drug delivery systems due to their high specific surface area, high drug loading capacity, NIR light- and pH-triggered drug release capabilities.¹⁶⁸ Layered double hydroxides were also reported as promising drug carriers due to their ability to incorporate large molecules as drugs.¹⁶² Other commonly known layered materials such as TMDs, transition metal oxides, black phosphorus and many more were also highly reported in

several reviews for their promising potential in drug delivery applications.¹⁶⁹⁻¹⁷¹ One commonly reported TMD is MoS₂ which have been proven with high potential for biomedical diagnosis and drug delivery.¹⁷² The transition metal oxide, MnO₂, can also act as intelligent cancer diagnostics and drug carriers for chemotherapy.¹⁷³

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PART I : Electrochemical Applications and Safety Studies of Layered Materials

Chapter 2

Cytotoxicity of Covalently Protected Layered Molybdenum Dichalcogenide

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2.1 Introduction

Transition metal dichalcogenides (TMDs) have been extensively used to form practical and highly useful two-dimensional (2D) nanomaterials.¹⁻⁴ Molybdenum disulfide (MoS₂) is a type of inorganic 2D TMD^{5,6} that has newly attracted wide and expanding interest due to their unique electronic,⁷ catalytic,^{8,9} and sensing^{10,11} properties. MoS₂ is commonly reported as a graphene analogue¹² and has a structure consisting of chemically bonded two-dimensional S–Mo–S nanosheets held together by van der Waals' interactions.^{13,14} Most 2D TMDs including MoS₂ have been more widely used in a form of single-layer nanosheets in various applications as it exhibit better photoluminescence properties,¹⁵ higher elastic properties^{16,17} and have highly improved electronic and structural properties.^{18,19} For example, it has been reported that with decreasing number of layers of a semiconducting TMD, we can easily tune the band gap of the material.²⁰ With easily tunable band gaps, these TMD single-layered nanosheets show promising performance in applications such as photodetectors, transistors and electroluminescent devices.²¹⁻²³ These single-layered nanosheets can be obtained through top-down chemical exfoliation of bulk MoS₂ with various intercalating agents.¹ The common intercalating agents reported for MoS₂ are lithium-containing compounds namely methyllithium (Me-Li), n-butyllithium (n-Bu-Li) and tert-butyllithium (t-Bu-Li).^{24,25} Amongst these, the more efficient exfoliation agents were reported to be via t-Bu-Li as well as n-Bu-Li.²⁶

Functionalization of MoS₂ has previously showed improvements in several existing properties such as biocompatibility,²⁷ electrochemical behavior,²⁸ catalytic performance,²⁹ and sensing abilities.³⁰ In this study, MoS₂ nanosheets were functionalized with the organic molecule, thiobarbituric acid (TBA).³¹ TBA acted as a nucleophilic thiol reagent to efficiently

functionalize the edges and basal planes of chemically exfoliated MoS₂ and more importantly, maintaining its metallic 1T character.³¹ TBA was selected for functionalization of MoS₂ amongst the other thiols available as it has a reactive sulphur atom, high tendency of further derivatization, economical and also, it lacks of odour.³¹ MoS₂-TBA was previously reported to have good aqueous dispersibility, improved hydrogen evolution reaction (HER) activity, enhanced electrocatalytic properties as well as promising potential in the oxidative detection of DNA nucleobases in solution.³¹ These improvements, as a result of functionalization, showed promising potentials in the future of functionalized TMD nanomaterials.

In view of these improvements, it is also important to understand the toxicity of MoS₂-TBA. Even though there are *in vitro* cytotoxicity studies of MoS₂ and other TMDs,^{32,33} no toxicity assessments have yet to be performed for MoS₂-TBA. It has been reported that the level of toxicity of a nanomaterial is influenced by several factors such as size, morphology, chemical composition and surface coating on the nanomaterial.^{34,35} With this, we aim to explore *in vitro* cytotoxicity of this chemically exfoliated MoS₂-TBA in comparison to non-functionalized chemically exfoliated MoS₂. Non-functionalized chemically exfoliated MoS₂ was proven to have low toxicity in previous studies.^{26,32} This study is essential as MoS₂-TBA has shown improved properties in various aspects³¹ and thus, has high possibility of commercial application in future. Due to their nanometer size, nanomaterials generally are able to enter the human body easily through inhalation, ingestion, skin penetrations or injections and possibly interact with intracellular structures and molecules.³⁶ Hence, MoS₂-TBA can also lead to health deterioration if they are released into the environment as a result of its utilization in commercial applications. With that, toxicity of MoS₂-TBA needs to be more understood before utilizing it in various applications in a larger scale. With the results obtained from this study, we hope to

pursue further research on mitigating the toxicity of the nanomaterials if it is indeed proven toxic to our health and the environment to a large degree.

This toxicity study will be conducted using two commonly used, well-established and simple cell viability assays which are (1) methylthiazolyldiphenyl-tetrazolium bromide (MTT) and (2) water-soluble tetrazolium salt (WST-8) assay. They both work following similar principles whereby tetrazolium compounds in the assay reagents are reduced in the presence of metabolically active cells to form formazan and generate dyed products.³⁷ The number of viable cells present after incubating with the nanomaterials is proportional to the colour intensity of formazan. Typically, two types of assays are used for a cytotoxicity study so that the results can be more reliable especially if results from both assays are coherent and complement each other. Human lung carcinoma epithelial cell line (A549) was chosen for this study as it is highly probable for nanomaterials to enter the lungs and interact with the body via inhalation through the respiratory tract from the environment. In addition, A549 cells are commonly used in several cytotoxicity studies of nanomaterials³⁸⁻⁴⁷ and hence, it allows easy comparison with other similar studies. The cytotoxicity results of MoS₂-TBA obtained from this study will be compared with that of MoS₂ which, although was already reported in several papers,^{26,32,48} was reassessed in this study using similar assays so as to ensure reliable comparison of results.

2.3 Results and Discussion

Material Characterization

Figure 1. Particle size distributions of MoS_2 and $\text{MoS}_2\text{-TBA}$ measured by Dynamic Light Scattering (DLS).

The particle size distribution measurements performed by dynamic light scattering (DLS) (**Figure 1**) show that particles from both MoS_2 and $\text{MoS}_2\text{-TBA}$ exhibit dominant peak at size 483.6 nm for MoS_2 and 414.8 nm for $\text{MoS}_2\text{-TBA}$. The range of sizes spread from 100 nm to 1000 nm for MoS_2 with a small amount of particles with large sizes of about 5560 nm. These large sizes may possibly be due to agglomeration of MoS_2 .⁴⁹ However, the range of sizes spread from 250 nm to 700 nm for $\text{MoS}_2\text{-TBA}$.

Zeta potential has been recently reported to efficiently analyse chemical modifications on the surface of nanomaterials.^{50,51} Hence, zeta potential of MoS_2 and $\text{MoS}_2\text{-TBA}$ was measured to quantify the surface charge of the nanomaterials. The zeta potential of MoS_2 and $\text{MoS}_2\text{-TBA}$ was found to be $-23.4 (\pm 0.9)$ mV and $-26.4 (\pm 0.7)$ mV respectively. From our results, $\text{MoS}_2\text{-TBA}$ possessed a more negative zeta potential than MoS_2 . This difference in surface charge may be due to the presence of carboxyl and amine functional groups on the surface of $\text{MoS}_2\text{-TBA}$.⁵¹

Cytotoxicity Studies

Herein, we studied how organic molecule functionalization influences the toxicity of MoS₂. *In vitro* cytotoxicity investigations were carried out by using two cell viability assays; MTT and WST-8 assays. The cell viability assay reagents were introduced after incubating A549 cells with different concentrations of nanomaterials, ranging from 0 to 100 µg/mL for 24 h. The presence of viable cells will reduce active tetrazolium reagent in WST-8 and MTT assay to form orange water-soluble formazan and purple insoluble formazan respectively. The insoluble formazan from MTT assay can be dissolved using combinations of detergent and organic solvents.⁵² The orange water-soluble formazan from WST-8 assay can be measured for absorbance at a wavelength of 450 nm while the dissolved purple formazan from MTT assay can be measured for absorbance at a wavelength of 570 nm. Since the amount of viable cells present after incubating with the nanomaterials is proportional to the colour intensity of formazan, absorbance is therefore proportional to the number of viable cells. The more accurate cytotoxicity data can be determined by normalizing with the absorbance reading of a control which is not exposed to nanomaterials. However, it has been reported that nanomaterials can interfere with the cell viability assays and give inaccurate results.⁵³ Hence, control experiments are conducted to take into account any interference due to the nanomaterials on the cell viability assays.

The first *in vitro* cytotoxicity assessment of MoS₂ and MoS₂-TBA was using water-soluble tetrazolium salt (WST-8) assay (**Figure 2A**). Before introduction of WST-8 assay reagent, the nanomaterials of different concentrations were incubated with A549 cells for 24 h. From **Figure 2A**, WST-8 assay showed a dose-dependent effect for both nanomaterials as the viability of the cells decrease when the concentration of nanomaterials increases. This

decreasing trend in cell viability is typical in cytotoxicity studies of materials which are toxic.⁵³ However, cell viability of cells exposed to MoS₂-TBA is higher as compared to the cell viability of cells exposed to MoS₂ at the tested concentrations. To illustrate, at the highest concentration of nanomaterials (100 µg/mL), 51% of cells which was exposed to MoS₂-TBA remained viable whereas only 31% of cells which was exposed to MoS₂ remained viable. At a lower concentration of 25 µg/mL, 83% of cells which was exposed to MoS₂-TBA remained viable whereas only 68% of cells which was exposed to MoS₂ remained viable. Hence, from WST-8 cell viability assay, we can conclude that MoS₂-TBA is less toxic than the non-functionalized MoS₂.

Figure 2. Percentage of cell viability of A549 cells that was exposed to different concentration of nanomaterials (MoS₂ and MoS₂-TBA) with **A**) WST-8 assay reagent and **B**) MTT assay reagent. The percentages are normalized to the readings of a control set that has 0 µg/mL of nanomaterials. The results presented a mean of at least three repeats with their respective $\pm$ standard deviations.

Following that, a second *in vitro* cytotoxicity assessment was conducted to ensure reliability of results obtained from WST-8 assay. The second cell viability assessment was conducted with methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. MTT assay was the first homogeneous cell viability assay developed for a 96-well plate that was suitable for high throughput screening.³⁷ Similar to WST-8 assay, the nanomaterials were introduced to A549 cells and incubated for 24 h before addition of MTT assay reagent. MTT forms insoluble formazan product in the presence of viable cells and the insoluble formazan can be dissolved using a combination of detergent and organic solvents. The results obtained from MTT assay (**Figure 2B**) showed a similar trend to that of WST-8 assay. From **Figure 2B**, a concentration-dependent trend was also seen for both nanomaterials when similar range of concentration (25, 50 and 100 $\mu\text{g/mL}$) was introduced. Similarly, the viability of cells that is exposed to MoS_2 -TBA was also seen to be higher than the viability of cells that was exposed to non-functionalized MoS_2 . To illustrate, at the highest concentration of nanomaterials (100 $\mu\text{g/mL}$), 68% of cells which was exposed to MoS_2 -TBA remains viable whereas only 49% of cells which was exposed to MoS_2 remains viable. At a lower concentration of 25 $\mu\text{g/mL}$, 75% of cells which was exposed to MoS_2 -TBA remained viable whereas only 53% of cells which was exposed to MoS_2 remained viable. Hence, from MTT cell viability assay, we can also conclude that MoS_2 -TBA is less toxic than the non-functionalized MoS_2 .

The slight differences in data obtained from WST-8 and MTT assays could be due to the differences in reducing abilities of the materials towards assay reagents. Both assays may also possess different sensitivities due to the presence of different active enzymes from the viable cells that is involved in the reaction. Despite the differences, both assays agree in concluding that the functionalized MoS_2 -TBA is less toxic than the non-functionalized MoS_2 . These results corroborate to previous reports on toxicity of functionalized MoS_2 . Yin et al. reported chitosan

(CS) functionalized MoS₂ (MoS₂-CS) being less toxic than non-functionalized MoS₂ which shows that functionalization of MoS₂ leads to lower toxicity.⁵⁴ Similarly, Liu et al. reported polyethylene glycol (PEG) functionalized MoS₂ (MoS₂-PEG) to have no obvious toxicity as compared to non-functionalized MoS₂.⁵⁵ A possible reason for the reduced toxicity is functionalization reduce the number of active edge sites that are capable of interacting with live cells and cause loss of viabilities. More precisely, edge sites of the nanosheets can possibly insert and cut into cell membranes leading to membrane stress and evidently, loss in cell viability.⁵⁶

Cell-free control experiments are necessary to evaluate the suitability of the assay for cell viability studies of a particular nanomaterial. It has been previously reported in several studies that some nanomaterials, including TMDs, can reduce the tetrazolium compound in the MTT and WST-8 assay reagents without the presence of viable cells.⁵⁷⁻⁵⁹ Such interferences may lead to false absorbance readings and therefore give false conclusions to the nanomaterials' cytotoxicity. Hence, to investigate the possibility of interference due to MoS₂ and MoS₂-TBA nanomaterials with MTT and WST-8 assay, similar concentrations of nanomaterials were tested under cell-free conditions with the same experimental conditions. In previous studies, an increase in absorbance values would show that the nanomaterial reduces the assay reagents and interfere with the absorbance measurements resulting in an overestimation of cell viability.³³ From the cell-free control experiment conducted (**Figure 3**), both WST-8 assay and MTT assays gave an increase in percentage absorbance as the concentration of nanomaterials increase. At the highest concentration (100 µg/mL), MoS₂ obtained a percentage absorbance of 345% with WST-8 assay (**Figure 3A**) and 275% with MTT assay (**Figure 3B**). MoS₂-TBA on the other hand obtained a percentage absorbance of 163% with WST-8 assay (**Figure 3A**) and 275% with MTT assay (**Figure 3B**).

Figure 3. Percentage absorbance obtained from MoS₂ and MoS₂-TBA in cell-free conditions with **A)** WST-8 assay reagent and **B)** MTT assay reagent. The percentages are normalized to the results of the control set that has 0 $\mu\text{g/mL}$ nanomaterials. The results presented a mean of at least three repeats with their respective $\pm$ standard deviations.

To further understand this increase in percentage absorbance, spectrum readings between wavelengths 350 nm and 550 nm (**Figures 4 and 5**) of both the control and cell viability experiments were conducted to further analyse any reduction capabilities of MoS₂ and MoS₂-TBA with the assay reagents. A presence of a peak indicates a reduction has occurred at that specific wavelength (**Figure 4B, 4D, 5B and 5D**). To illustrate, in **Figure 4B and 4D**, a peak was observed at wavelength of 450 nm when WST-8 assay reagent was introduced with the materials in the presence of cells. Similarly, in **Figure 5B and 5D**, a peak was observed at wavelength of 570 nm when MTT assay reagent was introduced with the materials in the presence of cells. However, no peaks were observed in the spectrum readings of the cell-free control experiments (**Figure 4A, 4C, 5A and 5C**). The absence of peaks of the control experiments at the respective wavelengths (450 and 570 nm) shows that no reduction of tetrazolium to formazan occurred due to the materials. We speculate that the high absorbance values were obtained possibly due to the presence of nanomaterials when absorbance measurements were read as the absorbance shifts to higher intensity with increasing concentration of materials. These results are supported by a previous study which reports that the absorbance spectrum shifts to a higher intensity with increasing layers of MoS₂.^{60,61} Hence, with the results obtained in the control experiments, a deduction of the absorbance readings of the control experiment (cell-free) from the cell viability absorbance readings was adopted so as to obtain a more accurate representation of cytotoxicity results in **Figure 2**.

Figure 4. Absorbance spectrum with WST-8 assay reagent of **A)** MoS₂ control (cell-free conditions) and **B)** MoS₂ cell viability and **C)** MoS₂-TBA control (cell-free conditions) and **D)** MoS₂-TBA cell viability between wavelengths of 350 nm to 550 nm.

Figure 5. Absorbance spectrum with MTT assay reagent of **A)** MoS₂ control (cell-free conditions) and **B)** MoS₂ cell viability and **C)** MoS₂-TBA control (cell-free conditions) and **D)** MoS₂-TBA cell viability between wavelengths of 490 nm to 650 nm.

2.4 Conclusion

With the expansion in applications of TMDs in various fields, MoS₂ serve as one of the leading TMDs used. Functionalization of MoS₂ to enhance its properties is not uncommon and MoS₂ functionalized with TBA is one example. MoS₂-TBA has been reported to have improvements in various aspects and the potential toxicity risks of MoS₂-TBA have yet to be understood. Our findings showed that MoS₂-TBA has lower toxicity as compared to MoS₂. This shows that functionalization can improve the quality of a material by reducing its toxicity. In this study, the results obtained are advantageous as MoS₂-TBA has been reported to have good aqueous dispersibility, improved hydrogen evolution reaction (HER) as well as show good potential in oxidative detection of DNA nucleobases in solution.³¹ Control experiments in the absence of cells were also conducted to evaluate any interference displayed by the nanomaterials towards the assay and it was found that there is an increase in absorbance as the concentration of nanomaterials increase. Previously, this correlation would be subjected to an increase amount of formazan as the nanomaterials were hypothesized to reduce the assay reagents in cell-free conditions.³³ However, further investigations in this study showed that these increase in absorbance did not result from increase amount of formazan. Formazan was not formed as a peak was absent from the spectrum readings of the control experiments. High absorbance values that were obtained from control experiments were hypothesized to possibly be due to interference due to the presence of nanomaterials during absorbance measurements. In conclusion, functionalization of MoS₂ with TBA has low toxicity and hence, is a safer material for usage in future potential applications.

2.5 Experimental Section

Chemicals

Bulk molybdenum disulfide (MoS_2) ($<2\ \mu\text{m}$) was obtained from Sigma–Aldrich (Singapore). Hexane was purchased from Lachner (Czech Republic). $t\text{-Bu-Li}$ in pentane (1.7 M) was obtained from Sigma–Aldrich (Czech Republic). Argon (99.9999% purity) was purchased from SIAD (Czech Republic). MTT assay reagent, N,N -dimethylformamide (DMF) and sodium dodecyl sulphate (SDS) was obtained from Sigma–Aldrich (Singapore). WST-8 assay reagent was obtained from Dojindo. 1% penicillin–streptomycin liquid and fetal bovine serum (FBS) was obtained from PAA Laboratories and Capricorn respectively. Dulbecco’s Modified Eagle medium (DMEM) and phosphate buffer solution (PBS) were obtained from Gibco. Glacial acetic acid was obtained from Schedelco.

Instrument

Dynamic light scattering (DLS) was carried out using Zetasizer Nano ZSP (Malvern, England). The DLS size and surface zeta potential measurements were performed at room temperature (20°C) using clear disposable zeta cells. Absorbance readings were measured by Thermo Multiskan GO (Thermo Fisher Scientific).

Synthetic methods of MoS_2 and $\text{MoS}_2\text{-TBA}$

MoS_2 used for this study was exfoliated by stirring MoS_2 bulk powder (3 g) in 20 mL of $t\text{-Bu-Li}$ (1.7 M) in pentane and stirred for 72 h in room temperature (25°C) under an argon environment. The Li-intercalated MoS_2 was obtained by suction filtration and washed a few times with hexane and dried over sodium. The obtained MoS_2 with intercalated Li was then

placed in water (100 mL) and centrifuged ($18,000 \times g$) to obtain exfoliation. Lastly, it is dried in a vacuum oven at 50°C for 48 h.³¹ MoS₂-TBA was obtained by functionalization of the chemically exfoliated MoS₂. The chemically exfoliated MoS₂ (25 mg, ~ 0.15 mmol) and thiobarbituric acid (30 mg, 0.20 mmol) were added to a reaction vial (4 mL) with a PTFE-lined cap, suspended in H₂O (1 mL), sonicated for 5 min at 37 kHz, and then heated at 75°C for 48 h with vigorous stirring. A grey mixture obtained after 48 h was then allowed to cool and then diluted with H₂O (3 mL) and then concentrated by centrifugation and washed with H₂O (thrice), followed with a 50/50 mixture of acetone:water (once), and finally with acetone (thrice). After each removal of the supernatant, the pellet resuspension and washing was aided by sonication for 5 min. Finally, the product was dried under vacuum overnight to yield 15 mg of dark grey powder.³¹

A549 cell culture

The human lung epithelial cell line (A549) was obtained from Bio-REV Singapore. It has typical cell cycle of 22 h. A549 were cultured in a cell culture medium which was made from a mixture of DMEM supplemented with 1% penicillin–streptomycin liquid and 10% FBS. The cells were incubated in 37°C under 5% CO₂. 5000 cells (100 μL) were seeded per well in a 96-well plate for 24 h.

MoS₂ and MoS₂-TBA introduction to A549

After cell seeding, the cell culture medium, as well as unattached cells in each well, was removed. The attached cells were then introduced to various concentrations of nanomaterials ranging from 0 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$ (100, 50, 25, and 0 $\mu\text{g/mL}$) and the remaining volume

was topped up to 100 μL with the cell culture medium. Cells with no nanomaterials were used as a negative control and cells with 10% dimethyl sulfoxide (DMSO) were used as a positive control to check for viability of the cells used. Thereafter, the cells and nanomaterials dispersions (100 μL per well) were incubated at 37°C and 5% CO_2 for another 24 h.

WST-8 assay

After 24 h of incubation with MoS_2 and MoS_2 -TBA, the cells were incubated with 10 μL of stock WST-8 solution at 37°C and 5% CO_2 for 1 h. After 1 h, its absorbance was measured at 450 nm. A spectrum reading of each concentration was also obtained.

MTT assay

After 24 h of incubation with MoS_2 and MoS_2 -TBA, the cells were incubated with 10 μL of the prepared MTT assay solution (5 mg/mL) per well at 37°C and 5% CO_2 for 4 h. After 4 h, the insoluble formazan formed was dissolved by 100 μL of a solubilisation solution which was made up of 16% (w/v) sodium dodecyl sulphate (SDS) dissolved in 40% (v/v) N,N-dimethylformamide (DMF) and 2% (v/v) glacial acetic acid. The plate was then agitated for 5 min to ensure complete solubilization. Finally, absorbance was measured directly from the 96-well plate at 570 nm.⁵² A spectrum reading of each concentration was also obtained.

WST-8 assay control experiment

Solutions of varying concentrations of MoS_2 and MoS_2 -TBA in cell culture media (100 μL) were incubated at 37°C and 5% CO_2 for 24 h. After 24 h of incubation with MoS_2 and MoS_2 -TBA, the solutions were incubated with 10 μL of stock WST-8 solution at 37°C and 5% CO_2

for 1 h. After 1 h, its absorbance was measured at 450 nm. A spectrum reading of each concentration was also obtained from the control experiment.

MTT assay control experiment

Solutions of various concentrations of MoS₂ and MoS₂-TBA in culture media (100 µL per well) were incubated at 37°C and 5% CO₂ for 24 h. After 24 h of incubation with MoS₂ and MoS₂-TBA, the solutions were incubated with MTT solution (5 mg/mL) and incubated at 37°C and 5% CO₂ for 3 h. After 3 h, the insoluble formazan formed was dissolved by 100 µL of a solubilization solution which was made up of 16% (w/v) sodium dodecyl sulphate (SDS) dissolved in 40% (v/v) dimethylformamide (DMF) and 2% (v/v) glacial acetic acid. The plate was then agitated for 5 min to ensure complete solubilization. Finally, absorbance was measured directly from the 96-well plate at 570 nm.⁵² A spectrum reading of each concentration was also obtained from the control experiment.

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Chapter 3

Electrochemical Applications and Cytotoxicity of
Layered Platinum Dichalcogenides

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3.1 Introduction

Oxygen reduction reaction (ORR) is the most crucial cathode reaction in fuel cells technology. The typical cathode platinum on carbon (Pt/C) catalyst shows the best electrocatalytic performance^{1,2} but severely suffer from high cost³, poor durability⁴ and possible toxicity.⁵ Particularly, Pt/C show poor stability on the catalytic activity, since the material is degraded easily by oxidation of the surface or by the dissolution of Pt/C at the cathode during potential cycling and consequent decreased in the active surface area.⁴ Hence, the search for new materials as electrocatalysts with high ORR capabilities is actively pursued. For instance, one strategy for enhanced ORR catalytic activity is using non-metal materials such as graphene related materials as a support for Pt nanostructures.⁶⁻⁸ Others explored on nitrogen-doped carbon materials as electrocatalysts for ORR.⁹ More recently, organic compounds like porphyrins and corroles were also reported to portray catalytic capabilities for ORR.¹⁰

Transition metal dichalcogenides (TMDs) have been researched as a promising class of layered materials for energy storage,^{11,12} electronics,^{13,14} catalysis,^{15,16} and biosensing^{17,18} applications.^{19,20} Specifically, TMDs have also been widely explored as catalyst for ORR.²¹ To date, molybdenum disulfide (MoS₂) is the most popular TMD studied for ORR.²²⁻²⁸ Recently, Rowley-Neale et al. reported on mass-producible two-dimensional MoS₂ impregnated screen-printed electrodes which has efficient electrocatalysis towards ORR.²⁷ Besides MoS₂, other TMDs have also been explored as reported by Eng et al. whereby the authors studied ORR capabilities of molybdenum diselenide (MoSe₂), tungsten disulfide (WS₂) and tungsten diselenide (WSe₂).²⁹ TMDs are attractive as they possess sizeable bandgaps³⁰, are naturally abundant³⁰ and exhibit favourable electrochemical properties²¹. TMDs consist of chemically bonded two-dimensional X-M-X layers held together by weak van der Waals' interactions (M: transition metal and X: chalcogen).³⁰ Another type of TMD with unique properties for energy

and electronic applications is platinum (Pt) dichalcogenides. Pt dichalcogenides are group 10 TMDs with chemical formula of PtX_2 ($\text{X} = \text{S}, \text{Se}, \text{Te}$).^{31,32} They are layered compounds with two-dimensional (2D) PtX_2 monolayers weakly stacked by van der Waals' interactions.^{31,32} Despite similarities in their bonding arrangements, many studies reported Pt dichalcogenides to have varying electronic properties. Specifically, in their bulk forms, PtS_2 is reported to be semiconducting, while PtSe_2 is semimetallic and PtTe_2 is metallic.^{31, 33} In addition, PtSe_2 was reported to be semiconducting when thinned to single-layer^{34,35} unlike its semimetallic bulk counterpart. With these unique electronic properties, Pt dichalcogenides possess promising properties for several applications.^{31,33-37}

Previously, Pt dichalcogenides have been reported to demonstrate competent electrocatalytic performance for hydrogen evolution reaction (HER) in comparison to Pt/C .^{33,35} HER is widely used reactions for generation of molecular hydrogen for the use in the fuel cells. It is therefore worthwhile to extend the research on electrocatalytic performance of Pt dichalcogenides to ORR, which is used to “burn” hydrogen (or alcohols) with oxygen in fuel cells. Herein, we studied electrocatalytic performance of Pt dichalcogenides (PtS_2 , PtSe_2 and PtTe_2) with regards to Pt/C for oxygen reduction reaction (ORR). From our electrocatalytic studies, we have found PtTe_2 to have comparable electrocatalytic performance with regards to Pt/C for ORR. This shows that PtTe_2 , like Pt/C , possess promising properties as electrocatalyst for ORR in industrial applications

Prior to any significant use of Pt dichalcogenides, it is essential that we investigate a detailed toxicity profile of these materials. Research on properties and applications of Pt dichalcogenides has been actively conducted in recent years.³¹⁻³⁹ However, research on the cytotoxicity of Pt dichalcogenides has yet to be pursued. Hence, a thorough cytotoxicity

investigation and comprehensive understanding of their toxicological effects are necessary as their usage may potentially increase over the years. Therefore, we also studied *in vitro* toxicity of Pt dichalcogenides. In addition, a comparative cytotoxicity study of Pt dichalcogenides with regards to platinum on carbon (Pt/C), a widely used electrocatalyst in fuel cell technology, is performed. These toxicity studies were conducted on two different cell lines namely, human lung carcinoma epithelial cell line (A549) and human lung epithelial cell line (BEAS-2B). Lung cell lines are chosen as the probability for nanomaterials to enter the lungs via inhalation is high. The viabilities of the cells incubated by Pt dichalcogenides will then be quantified by water-soluble tetrazolium salt (WST-8) assay, a fast, efficient and well-known assay to measure cell viability. From our toxicity studies, we have found toxicity of Pt dichalcogenides to be significantly lower than the toxicity of Pt/C. In short, PtTe₂ demonstrated promising properties to replace Pt/C as catalyst for ORR with much lower toxicity.

3.2 Results and Discussion

Characterization

Characterization is necessary to evaluate successful synthesis of the desired Pt dichalcogenides namely, PtS₂, PtSe₂ and PtTe₂. In addition, differences in physicochemical properties of nanomaterials for example, morphology, size and chemical composition can highly influence the way nanomaterials interact with the cells.⁴⁰ Hence, a detailed characterization of nanomaterials is important in order to determine an accurate toxicological profile for the nanomaterial. Several characterizations of the Pt dichalcogenides were performed prior to conducting the proposed electrocatalytic and cytotoxicity assessments.

The synthesized Pt dichalcogenides were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), high-resolution transmission electron microscopy (HRTEM), selected area diffraction (SAED) and dynamic light scattering (DLS).

Figure 1. Scanning electron microscopy (SEM) images of Pt dichalcogenides (PtS₂, PtSe₂ and PtTe₂) in high magnification (x 10 000). The scale bar represents 1 μm.

Scanning electron microscopy (SEM) images (Figure 1) and transmission electron spectroscopy (TEM) images (Figure 2) from the synthesized Pt dichalcogenides revealed layered structures for all three materials. SEM images also showed large polygonal stacks up to micrometres sized typical of bulk form. TEM images (Figure 2) showed the layered flakes of PtTe₂ and PtSe₂ with a particles size around 1 μm . The selected area electron diffractions (SAED) (Figure 2) of PtTe₂ and PtSe₂ flakes provide evidence for the hexagonal in-plane structure corresponding to $P\bar{3}m1$ space group.³³ The HR-TEM image (Figure 2) shows perpendicular view on (00l) plane and lattice fringes corresponding to (h00), (0k0) and (hk0) planes. On PtSe₂ are visible lattice fringes with distance of 0.33 nm corresponding to (100) plane and on PtTe₂ are visible lattice fringes with distance of 0.29 nm corresponding to (011) plane. These results are in good agreement with lattice parameters of corresponding phases.³³ On the other hand, the TEM, HR-TEM and SAED of PtS₂ (Figure 2) suggest that it has an amorphous structure.

Figure 2. TEM images with SAED (inset) and HR-TEM of Pt dichalcogenides.

EDS from TEM (Figure 3) and SEM (Figure S1) provides an elemental composition and mapping of the elements that are present over the whole material. From the EDS maps obtained in Figure 3 and Figure S1, the platinum and chalcogen elements are shown to be well distributed on the surface of the nanomaterials. Also, the chalcogen-to-metal ratio were obtained from EDS and are presented in Table S1. The chalcogen-to-metal ratio of PtS₂, PtSe₂ and PtTe₂ were found to be 1.8, 2.0 and 2.1 respectively. These ratios are similar to the chalcogen-to-metal ratio reported in a study by Chia *et al.*³³

Figure 3. Transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS) images of Pt dichalcogenides (PtS_2 , PtSe_2 and PtTe_2).

X-ray photoelectron spectroscopy (XPS) was employed to determine their elemental surface compositions and chemical bonding. Figure S2 shows the survey scan of Pt dichalcogenides. Besides O and Si present in the sample from the substrate, we can identify Pt and the chalcogens in the corresponding scan spectra. In the high resolution XPS (Figure 4), the predominant Pt oxidation states for the three Pt dichalcogenides vary. The predominant oxidation state for PtS_2 is Pt(IV) as depicted by Pt $4f_{7/2}$ and Pt $4f_{5/2}$ binding energies at 73.4 and 76.8 eV but the

predominant oxidation states of PtSe₂ and PtTe₂ is Pt(II) as depicted by Pt 4f_{7/2} and Pt 4f_{5/2} binding energies at 72.8 and 76.1 eV for PtSe₂ and 72.1 and 75.5 eV for PtTe₂.⁴¹ The S chalcogen in PtS₂, existing as S(-II), were largely oxidized into HSO₄⁻ and SO₄²⁻ and was seen as a broad S 2p signal with binding energy of 167.8 eV.⁴² However, S²⁻ was still present in lower intensity at binding energies of 161.8 and 162.9 eV which corresponds to S²⁻ 2p_{3/2} and 2p_{1/2} peaks respectively.³³ The Se chalcogen in PtSe₂ exist largely as Se(-II) as depicted by 3d_{7/2} and 3d_{5/2} binding energies at 54.2 and 55.1 eV.³³ Also, as seen in the Te 3d XPS spectra of PtTe₂, the signals at 574.6 and 585.0 eV corresponds to 3d_{5/2} and 3d_{3/2} binding energies of Te(IV) and the signals at 572.0 and 582.4 eV corresponds to 3d_{5/2} and 3d_{3/2} binding energies of Te(0).⁴³

The X-ray diffraction (XRD) was used to evaluate crystal structure and phase purity of the Pt dichalcogenides (Figure S3). The X-ray diffractograms of PtSe₂ and PtTe₂ exhibit single phase composition space group ($P\bar{3}m1$) and non-additional phases are observed, both showing preferential orientation of 001 that are typical for layered structures. PtS₂ on the other hand, exhibit an amorphous structure.³³

The particle size distribution measurements performed by dynamic light scattering (DLS) (Figure S4) show that particles from all Pt dichalcogenides exhibit dominant distribution peak with sizes in the range of 50 - 300 nm. In addition, PtTe₂ exhibited a small peak at 5560 nm which most probably results from large particles due to agglomeration.

Figure 4. High resolution X-ray photoelectron spectra (XPS) of Pt 4f and chalcogen (S 2p, Se 3d and Te 3d) regions of Pt dichalcogenides.

Oxygen reduction reaction (ORR)

In previous studies, Pt dichalcogenides exhibit different electronic properties that range from semiconductor (PtS_2 and PtSe_2) to metallic (PtTe_2)^{31,33,44}. Taking into account these differences, we performed ORR electrocatalysis with Pt dichalcogenides and we presented linear sweep voltammetry (LSV) measurements in Figure 5A. The polarization curves of Pt dichalcogenides and Pt/C towards the negative potential direction show that the cathodic current decrease at relative low potential due to the direct 4-electron reduction pathway from O_2 to H_2O in aqueous solution.¹⁰

Interestingly, the ORR performance of PtTe_2 (orange line) is better as compared to its other Pt dichalcogenides counterparts (PtS_2 and PtSe_2). In addition, the ORR performance of PtTe_2 is comparable to that of Pt/C (green line). The ORR catalysis was confirmed using KOH solution purged with N_2 gas (dash lines) where the ORR peaks are absent. The same trend is observed when we compare the onset potentials in Figure 5B. The onset potential of PtTe_2 is close to the onset potential of Pt/C with potential difference of 0.018 V. This high ORR performance of PtTe_2 probably is due to its metallic nature as reported before.^{31, 33}

The stability of PtTe_2 and Pt/C as an ORR catalyst was also evaluated. 100 cyclic voltammograms (CV) were recorded in a negative window potential from -50 to -250 mV. ORR performances of PtTe_2 and Pt/C were measured before and after the CV cycling and the results are represented in Figures 5C and 5D for Pt/C and PtTe_2 respectively. Interestingly, after the cycling the onset potential decreased by 28 mV for Pt/C and 34 mV for PtTe_2 demonstrating the high ORR stability of PtTe_2 that is similar to ORR stability of the control material, Pt/C.

Figure 5. Oxygen reduction reaction (ORR). **A)** Linear sweep voltammogram (LSV) on Pt dichalcogenides, Pt/C and bare GC in ambient (solid lines) and purged (dashed lines) conditions. **B)** Onset potentials for ORR on Pt dichalcogenides, Pt/C and bare GC using the potential at which 10% of the maximum current density is achieved. Error bars correspond to standard deviations based on triplicate measurements. **C)** and **D)** corresponds to stability test of Pt/C and PtTe₂ respectively. The respective LSVs are recorded before and after performing 100 cycles of cyclic voltammetry (CV) in a potential window of -50 to -250 mV. Potentials are with respect to the Ag/AgCl reference electrode. Bare GC stands for the unmodified bare glassy carbon electrode.

Cytotoxicity assessments

Next, we studied the *in vitro* cytotoxicity of platinum (Pt) dichalcogenides in comparison to Pt on carbon (Pt/C). Cytotoxicity studies were conducted by assessing the viability of cells after 24 hours exposure to nanomaterials. Cell viability was measured using water-soluble tetrazolium salt (WST-8) cell viability assay, a common cell viability assay that assesses the presence of mitochondrial activity, which correlates to the health of the cell. In the presence of healthy cells, the tetrazolium reagents in WST-8 assay will get reduced to generate coloured formazan dyes whose colour intensity reflects the number of viable cells present.⁴⁵

Human lung carcinoma epithelial cell (A549) and human lung epithelial cell (BEAS-2B) were chosen because they are the most suitable cells, taking account the route of exposure. Cell lines that were obtained from the human lungs are the most relevant because the possibility of nanomaterials entering the human lungs via inhalation is high.⁴⁶ Since respiratory tract is a primary route of exposure to inhaled particles, lung-related cell lines are therefore suitable models of cytotoxicity studies on nanomaterials. Hence, it would be a good representative of how inhalation of these nanomaterials from the environment can affect our health. In addition, A549 cells are common cells used in several cytotoxicity studies of nanomaterials. By using this cell line, it allows easy comparison with other cytotoxicity studies⁴⁷⁻⁵⁶ and thus, gives a more accurate evaluation of cytotoxicity profiles. On the other hand, BEAS-2B cells are a more accurate representative of cells in which the inhaled nanomaterials can interact with as they are non-cancerous human cells obtained from healthy human lungs. Despite their differences, both cell lines should eventually give results with the same trend. Results obtained from these two cell lines can confirm the nanomaterials' overall cytotoxicity profile more effectively if they both present the same toxicity trend.

Figure 6. Cell viability of **A)** A549 and **B)** BEAS-2B cells measured by WST-8 assay after 24 hours exposure to varying amounts of nanomaterials. The percentages are normalized to the data obtained from the control set that was exposed to 0 $\mu\text{g/mL}$ nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

The results obtained from nanomaterials exposure to A549 cells are presented in Figure 6A. When comparing cytotoxicity of Pt dichalcogenides in Figure 6A, PtS₂ showed minimal toxicity across all concentrations while PtSe₂ and PtTe₂ showed dose dependent toxicity across a varying concentration of nanomaterials. Upon introduction of PtS₂ to cells, percentage of cell viability remains above 90% across all concentrations, denoting PtS₂ as the least toxic nanomaterial. The dose dependent toxicity of PtSe₂ is only evident at higher concentrations (above 25 µg/mL) of nanomaterial but retained at least 40% of viable cells upon introduction of nanomaterials at all concentrations. On the other hand, 50% of cells were no longer viable after introducing 25 µg/mL of PtTe₂. A low cell viability of 14% was obtained upon introduction of 200 µg/mL of PtTe₂, denoting PtTe₂ as the most toxic Pt dichalcogenide.

The results obtained from nanomaterials exposure to BEAS-2B cells are presented in Figure 6B. The results obtained with BEAS-2B cells presented similar trend to that obtained with A549 cells in Figure 6A. Similar to Figure 6A, PtS₂ showed negligible toxicity across all concentrations while PtSe₂ and PtTe₂ showed dose dependent toxicity across the varying concentration of nanomaterials. Upon introduction of PtS₂ to the cells, cell viability remained above 80% across all concentrations, denoting PtS₂ as the least toxic nanomaterial. Similar to results obtain from A549 cells, more 40% of cells remained viable upon introduction of PtSe₂ at all concentrations. On the other hand, more than 50% of cells were no longer viable upon introduction of 50 µg/mL of PtTe₂ and less than 10% of the cells remained viable with 200 µg/mL of PtTe₂, denoting PtTe₂ as the most toxic Pt dichalcogenide.

We hypothesize that the mechanism of toxicity of Pt dichalcogenides and Pt/C would be due to the production of excess reactive oxygen species (ROS). ROS are essential but can be detrimental in high levels. High ROS levels may lead to oxidative stress in the cells and can

damage cells by peroxidizing lipids, altering proteins and disrupting DNA.⁵⁷ Despite the slight discrepancy in cell viability percentages, cell viability trends of Pt dichalcogenides tested in both cell lines are similar. The confirmed trend that can be concluded from both cell lines is that the toxicity of Pt dichalcogenides increase down the chalcogen group. This means that toxicity of Pt dichalcogenides follow the trend of: $\text{PtTe}_2 > \text{PtSe}_2 > \text{PtS}_2$. This trend is supported by a similar study by Latiff *et al.* in the study of cytotoxicity of exfoliated layered vanadium dichalcogenides whereby toxicity of vanadium dichalcogenides increases as the chalcogen change down the group.⁴⁷

One possible reason for the differences in toxicity profile can be the difference in metallic character of the materials. As reported in many studies, PtS_2 have been found to be semiconducting, PtSe_2 to be semimetallic and PtTe_2 to be metallic.^{31,33,44} Some studies also reported PtSe_2 to have semiconducting properties.³⁴ This shows an increase in metallic character down the group. Since it was found that toxicity level increase down the chalcogen group, it is possible that toxicity level of the material increases with its metallic character.

One interesting observation from these results is that PtTe_2 has the best performance as electrocatalyst for ORR electrocatalysis but is found to be the most toxic Pt dichalcogenide. Hence, it is worthwhile to compare the toxicity of PtTe_2 with regards to Pt/C, the best reported electrocatalyst to date³¹. With this, a toxicity study in comparison with Pt/C was conducted. Pt/C was also introduced to both A549 cells (Figure 6A) and BEAS-2B cells (Figure 6B). In both cell lines, Pt/C was found to be highly toxic even in low concentrations. Only 30% of A549 cells remained viable when 12.5 $\mu\text{g/mL}$ of Pt/C was introduced and less than 50% of BEAS-2B cells remained viable when 25 $\mu\text{g/mL}$ of Pt/C was introduced. In both cell lines, no cells remained viable after 50 $\mu\text{g/mL}$ of Pt/C was introduced (as shown by the red circle). A control experiment on carbon was conducted with both cell lines so as to eliminate toxic effects

due to carbon in Pt/C (Figure S5 and S6), and it was observed that 48% of the cells remained viable even when a high concentration of 200 $\mu\text{g/mL}$ was introduced. This proves that carbon is not the main source of toxicity in Pt/C.

In summary, we can further conclude that the toxicity Pt dichalcogenides and Pt/C adheres to a trend of $\text{Pt/C} > \text{PtTe}_2 > \text{PtSe}_2 > \text{PtS}_2$. Possibly, the synergistic effect of platinum bonded to chalcogens may have reduced the toxicity level of the materials. In previous studies from our group, the toxicity of other TMDs have been studied, and these materials demonstrated relatively low toxicities^{57,58}. This shows that despite chalcogens being toxic itself⁵⁹⁻⁶¹, the synergistic interaction of a transition metal with chalcogen (S, Se and Te) may reduce the overall toxic effect of the TMD compound as compared to the individual chalcogen. Hence, we hypothesize that Pt dichalcogenides has lower toxicity than pure Pt/C due to the effect of synergistic interaction of Pt with the respective chalcogens.

To deeper understand the obtained results, we also compared cytotoxicity of Pt dichalcogenides with the toxicity of other materials reported in the past which possess competent ORR catalytic activity. This comparison is presented in Table S2. From the comparison, PtTe_2 is generally less toxic than Pt/C but more toxic than MoS_2 ⁵⁸, another TMD reported for ORR²⁵⁻²⁸. On the other hand, some graphene derivatives are also reported as competent ORR catalyst.⁶²⁻⁶⁵ However, comparing toxicity of PtTe_2 with toxicity of graphene derivatives, PtTe_2 is more toxic than fluorographene⁶⁶ but less toxic than graphene oxide (Hummers).⁵⁵ However in terms of electrocatalytic performance, PtTe_2 shows superior ORR performance at lower onset potential as compared with MoS_2 . While in comparison with graphene related materials, the ORR performance is similar. With this, Pt dichalcogenides can be a promising alternative as

ORR electrocatalyst as they are less toxic materials as compared to Pt/C denoting them as a safer class of materials in clean energy reactions such as HER and ORR.

Cell-free control experiments

Cell-free control experiments are important to evaluate any forms of interference originating from the assay as well as from the nanomaterials. It was reported in many studies that some nanomaterials can interfere with a cell viability assay by reducing the tetrazolium component in the assay into formazan even in the absence of viable cells.^{48,49} Other reports also stated that some nanomaterials can have absorbance and these additional factors have to be thoroughly evaluated so as to avoid obtaining false results leading to false conclusions.⁶⁷ Hence, to investigate any of such interference, cell-free control experiments were conducted and the results are presented in Figure 7.

The cell-free control experiments are conducted in similar conditions to that of the cell viability assessments but in the absence of cells. Specifically, similar concentrations of the nanomaterials were incubated (37°C under 5% CO₂) in cell culture media with no cells and tested with WST-8 assay after 24 hours. WST-8 assay assesses mitochondrial activity in which the tetrazolium reagents in the assay will be reduced, in the presence of metabolically active cells, to generate coloured formazan dyes whose colour intensity reflects the percentage of viable cells present.⁴⁵ Hence, in the absence of cells, formazan dyes should be also absent. However, the results in Figure 7 show is an increase in absorbance with increase concentration of nanomaterials for PtSe₂ and PtTe₂. This increasing trend is absent in PtS₂. The increase in absorbance can be contributed by various factors. One factor could be due to reduction of tetrazolium salt by nanomaterials. However, this factor is dismissed after further evaluating the absorbance spectra of the respective nanomaterials (Figure S7). In the presence of formazan, a

peak would be observed at 450 nm as seen in Figure S7A-S7C. However, this specific peak is absent in spectrum readings of the cell-free control experiments (Figure S7D-S7F) which implies that no formazan was formed. This means that the nanomaterials are not able to reduce tetrazolium salt in WST-8 assay reagent. However, as the concentration of the nanomaterials increase, the absorbance shifted to higher values for PtSe₂ and PtTe₂ (Figure S7E and S7F). This shift in absorbance is absent for PtS₂ (Figure S7D). This shows that the nanomaterials directly interfere with absorbance readings and cause an increasing shift in absorbance values at with increasing concentration of nanomaterials.

Hence, these absorbance results obtained in Figure 7 were subtracted from the both cell viability assessments readings in Figure 6. The subtraction of the absorbance readings of the control experiments from the absorbance readings of the actual cell viability assessments eliminates any interference present due to absorbance shifts in cell viability assessments.

Figure 7. Percentage absorbance of varying concentration of nanomaterials in cell-free conditions measured by WST-8 assay. The percentages are normalized to the readings of the control set that was exposed to 0 µg/mL nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

3.3 Conclusion

In conclusion, we have explored the ORR electrocatalytic performance and cytotoxicity properties of Pt dichalcogenides and compared them with platinum on carbon (Pt/C). From our results, the ORR electrocatalytic performance of PtTe₂ was found to be better than that its other Pt dichalcogenide counterparts (PtS₂ and PtSe₂) and interestingly, the ORR performance of PtTe₂ is very similar to that of Pt/C, the best reported electrocatalyst for ORR. In addition, from our cytotoxicity studies, we have found that toxicity of PtTe₂ is significantly lower than the toxicity of Pt/C. Our cytotoxicity results showed that the toxicity of Pt dichalcogenides were significantly lower than the toxicity of Pt/C, adhering to a trend of Pt/C>PtTe₂>PtSe₂>PtS₂. This trend shows that the toxicity level of Pt dichalcogenides increases down the chalcogen group and we hypothesize that it may be due the increase in metallic character of the materials. Thus, we have demonstrated that PtTe₂ have promising properties to possibly replace Pt/C as an electrocatalyst for ORR as it possesses similar electrocatalytic performance to Pt/C and significantly lower toxicity. In overall, our studies can allow better understanding of the electrocatalytic performance and toxicological profiles and of Pt dichalcogenides in comparison to Pt/C to aid future mass application and commercialisation in clean energy reactions such as ORR.

3.4 Experimental Section

Nanomaterials

The ammonium hexachloroplatinate (IV) and chloroplatinic acid hexahydrate was obtained from Safina, Czech Republic. The sulfur (99.999%) was obtained from STREM, Germany. Selenium (99.999%) and tellurium (99.999%) were obtained from Chempur, Germany. Hydrogen (99.9999%) and hydrogen sulfide gas (99.8%) were obtained from SIAD, Czech Republic. Carbon nanopowder and platinum on graphitized carbon were both obtained from Sigma Aldrich.

Cell Viability Assays and Culture Media

WST-8 assay reagent was obtained from Dojindo. Fetal bovine serum (FBS) and 1% penicillin-streptomycin liquid were purchased from PAA Laboratories and Capricorn respectively. Dulbecco's Modified Eagle medium (DMEM) and phosphate buffer solution (PBS) of pH 7.2 were purchased from Gibco.

Instruments

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were carried out using a Jeol 7600F instrument (Jeol, Japan). X-ray photoelectron spectroscopy (XPS) measurements were carried out using Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). High-resolution transmission electron microscopy (HR-TEM) data were obtained with the EFTEM Jeol 2200 FS microscope (Jeol, Japan). X-ray diffraction (XRD) was performed using a Bruker D8 Discoverer powder diffractometer with parafocusing Bragg – Brentano geometry and Cu K α radiation. Dynamic light scattering (DLS) measurements were conducted with Zetasizer Nano ZS from Malvern Instruments (England). Electrochemical ORR measurements were conducted using a μ Autolab

type III electrochemical analyzer (Eco Chemie, The Netherlands). Absorbance readings were measured by Thermo Multiskan GO (Thermo Fisher Scientific).

Synthesis methods of Pt dichalcogenides

Platinum dichalcogenides was synthesized from their respective elements in evacuated quartz glass ampoules (100 mm long, 18 mm external diameter, wall thickness of 3 mm). Platinum was obtained from the reduction of ammonium hexachloroplatinate(IV) by hydrogen at 500 °C for 1 hour. The platinum metal and chalcogen used were mixed in stoichiometric amounts in which 10 wt% of S and 3 wt% excess of Se and Te over stoichiometry corresponding to 2 g of platinum dichalcogenide were mixed. The quartz ampoule was melt-sealed using oxygen-hydrogen torch at a pressure of 1×10^{-5} mbar. The ampoule with PtTe₂ was heated at 1000 °C for 48 hours and finally at 1150 °C for 1 hour. The heating rate was 10 °C min⁻¹ and the cooling rate 1 °C min⁻¹. The ampoule with PtSe₂ was heated at 800 °C for 48 hours and subsequently at 1270 °C for 1 hour. The heating rate was also 10 °C min⁻¹ and the cooling rate was 1 °C min⁻¹. PtS₂ was prepared by reaction of hexachloroplatinic acid with hydrogen sulfide. Chloroplatinic acid hexahydrate of 1 g was dissolved in 50mL of water and acidified with hydrochloric acid at pH 2. Hydrogen sulfide gas was bubbled into the solution for 8 hours. The black precipitate of PtS₂ was separated by suction filtration and repeatedly washed with water. Finally, PtS₂ was dried in vacuum oven for 48 hours.

Cell Culture

Human lung epithelial cell line (A549) was obtained from Bio-REV Singapore and have a typical cell cycle of 22 hours. Human bronchial epithelial cell line (BEAS-2B, CRL-9609) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells

were cultured in a cell culture medium which is made up of a mixture of DMEM with 10% FBS and 1% penicillin-streptomycin liquid, in an incubator of 37°C under 5% CO₂. 5000 cells were seeded per well (100µL) in a 96-well plate for 24 hours.

Nanomaterials introduction to cells

PtS₂, PtSe₂ and PtTe₂ samples were prepared in concentrations of 1 mg/mL in ultrapure water and subjected to ultrasonication for 3 hours to attain well-dispersed suspensions. Prior to each test, the samples undergo ultrasonication again for 10 minutes to maintain the homogeneous dispersion of the sample. After incubating the seeded cells for 24 hours, the cell culture medium per well was removed. The cells were then introduced to various concentrations of nanomaterials ranging from 0 µg/mL to 200 µg/mL. Cells with no nanomaterials were used as a negative control and cells with 10% DMSO were used as a positive control. The cells and nanomaterials dispersion (100 µL per well) were incubated at 37°C under 5% CO₂.

WST-8 cell viability assay

After incubating the cells with the nanomaterials for 24 hours, 10µL of stock WST-8 reagent was introduced and further incubated (37°C and 5% CO₂) for 1 hour. After 1 hour, absorbance measurement at 450 nm and spectrum absorbance measurement (400 nm to 500 nm) of every concentration were obtained.

WST-8 control experiment

Nanomaterials of varying concentrations (0 to 200 µg/mL) were incubated (37°C and 5% CO₂) in cell culture media for 24 hours. After 24 hours, similar to cell viability assessment, 10µL of stock WST-8 reagent was introduced and further incubated for 1 hour. After 1 hour, absorbance

measurement at 450 nm and spectrum absorbance measurement (400 nm to 500 nm) of every concentration were obtained.

Oxygen reduction reaction

Oxygen reduction reaction (ORR) measurements were performed using linear sweep voltammetry at scan rates of 5 and 2 mV s⁻¹, respectively, in 0.1 M KOH electrolyte. The measurements were carried out using a three-electrode system (a glassy carbon working electrode modified with the material of interest, platinum auxiliary electrode & Ag/AgCl reference electrode) in a glass cell at room temperature.

3.5 Supporting Information

Figure S1. EDS images and mappings of Pt dichalcogenides (PtS₂, PtSe₂ and PtTe₂). The scale bar represents 10μm.

Table S1. Elemental compositions of Pt dichalcogenides and its chalcogen to metal ratio obtained from EDS.

Material	Pt	S	Se	Te	Chalcogen: Metal
PtS ₂	64.48	35.52	-	-	1.8
PtSe ₂	66.54	-	33.46	-	2.0
PtTe ₂	68.03	-	-	31.97	2.1

Figure S2. Wide-scan X-ray photoelectron survey spectra of Pt dichalcogenides.

Figure S3. X-ray diffraction (XRD) of Pt dichalcogenides.

Figure S4. Particle size distributions of Pt dichalcogenides measured by dynamic light scattering (DLS).

Figure S5. Cell viability of A549 cells measured by WST-8 assay after 24 hours exposure to varying amounts of carbon nanopowder. The percentages are normalized to the data obtained from the control set that was exposed to 0 µg/mL nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

Figure S6. Cell viability of BEAS-2B cells measured by WST-8 assay after 24 hours exposure to varying amounts of carbon nanopowder. The percentages are normalized to the data obtained from the control set that was exposed to 0 µg/mL nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

Figure S7. Absorbance spectrum with WST-8 assay reagent of **A)** PtS₂, **B)** PtSe₂ and **C)** PtTe₂ cell viability and **D)** PtS₂, **E)** PtSe₂ and **F)** PtTe₂ control (cell-free conditions) of varying concentrations between wavelengths of 400 nm and 500 nm.

Table S2. Percentage of cell viability (determined from WST-8 assay) of various 2D nanomaterials with competent ORR performance.

Materials		Cell Viability	ORR onset potential	Reference
PtTe₂		40 % (50 µg/ml of materials exposed to A549 cells)	-0.14 V (vs Ag/AgCl)	This work
Pt/C		0 % (50 µg/ml of materials exposed to A549 cells)	-0.12 V (vs Ag/AgCl)	This work
MoS₂		95% (50 µg/ml of materials exposed to A549 cells)	-0.78 V (vs Ag/AgCl)	57,29
Fluorographene	CHF [24+24h:5+12bar]	-	+0.087V (vs NHE)	62
	F-G596	55% (50 µg/ml of materials exposed to A549 cells)	-	65
Graphene Oxide	Hu-ErGO	-	-0.17V (vs Ag/AgCl)	64
	GO-Hu	30% (35 µg/ml of materials exposed to A549 cells)	-	55

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Chapter 4

Electrochemical Applications and Cytotoxicity of Layered Carbide and Boride: MAX and MAB Phases

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4.1 Introduction

The declining abundance of non-renewable fossil fuels has led to a more intensified search for alternative clean, renewable and efficient energy production sources. One strategy is to search for cost effective, efficient and clean energy generation methods such as fuel cells.¹ Electrochemical splitting of water molecules are able to generate hydrogen gas which can be harnessed as fuel in fuel cells. However, these electrochemical reactions are heavily dependent on expensive catalysts, such as noble metals to proceed efficiently. Hence, tremendous research has been performed to seek alternatives for these expensive catalysts.¹

Layered materials²⁻⁴ such as transition metal dichalcogenides (TMDs),⁵⁻⁸ black phosphorus (BP)⁹⁻¹¹ and heavy pnictogens¹² have been reported as alternative materials due to abundance and promising performances. For instance, Group 6 TMDs, such as MoS₂, and WS₂, have been widely reported as excellent low-cost, abundant and non-polluting catalyst for hydrogen evolution reaction (HER) and oxygen reduction reaction (ORR).^{13,14} First row TMD nanoparticles were also reported to have exceptional performances as non-precious metal catalysts for HER.¹⁵ More recently, BP^{9,11} and heavy pnictogens,¹² specifically As, Sb and Bi, displayed promising electrocatalytic performances for HER, ORR and oxygen evolution reaction (OER). Beyond these, there exists another family of layered materials known as MAX and MAB phases which have yet to be explored.

MAX and MAB phases, also known as ceramic nanolaminates, have been reported to possess excellent thermal and electrical conductivities, elastic stiffness, chemical and thermal-shock resistance, damage tolerance and are readily machinable.¹⁶⁻¹⁹ MAX phases are layered ternary transition metal carbides and nitrides with a general formula of M_{n+1}AX_n where n: 1, 2 or 3, M: early transition metal, A: III_A and IV_A group element and X: C or N.^{20,21} They possess

hexagonal crystal structures with $P6_3/mmc$ symmetry with their near-closed-packed M and X layers interleaved by A atoms.^{20,21} Unlike graphite and TMDs where the layers are bonded by weak van der Waals interactions, the bonds between the layers of MAX phases are stronger and broken by shear force or mechanical means.²⁰ Therefore, they can undergo selective etching and exfoliation to form another group of materials known as MXenes which are two dimensional M-X monolayer structures. These materials have recently been reported to possess promising electrocatalytic properties for clean energy applications.^{22,23} MAB phases are layered ternary transition metal borides with several variable formulas including $MAIB$, M_2AlB_2 , M_3AlB_4 and M_4AlB_6 where M: transition metal, A: III_A and IV_A group element and B: boron.^{16,17} They possess orthorhombic crystal structure with varying space groups from $Cmcm$ for $MAIB$, to $Cmmm$ for M_2AlB_2 and M_4AlB_6 , and $Pmmm$ for M_3AlB_4 .¹⁶ The M-A bond is metallic while M-X/B bond portrays a mixed covalent/metallic/ionic character.²¹ The synthetic illustration and chemical structures of these compounds are illustrated in **Scheme 1**.

Scheme 1. Synthetic illustration and chemical structures of respective MAX and MAB phases.

Recent works related to MAX and MAB phases primarily focused on several fundamental properties and characteristics but less on their potential applications.^{16-21,24} Since several layered materials have shown promising properties in electrochemical and electrocatalytic applications, it is worthwhile to expand such studies to MAX and MAB phases. Herein, we explored their fundamental electrochemical properties namely inherent electrochemistry and heterogeneous electron transfer (HET) rate. Following that, we investigated their potential in electrochemical sensing and electrocatalytic applications- specifically towards energy applications such as HER, ORR and OER.

In addition, prior to large scale applications, understanding toxicological properties of emerging materials are important to evaluate their health safety and be informed of potential risks upon exposure to human and the environment.²⁵⁻²⁷ Toxicity of commonly known layered materials such as graphene and its derivatives, TMDs and pnictogens towards human cells have been widely studied.²⁸⁻³⁹ For instance, the toxicological properties of graphene and graphene oxide (GO) were reported to be dependent on the concentration exposed, fabrication method adopted, quantity of oxygen groups and particle sizes.²⁷ They possess higher toxicity than Group 6 TMDs such as MoS₂ and WS₂.³⁰ In addition, pnictogens, such as phosphorus allotropes (BP) and heavy pnictogens (As, Sb and Bi), were found to have varying toxicities. BP is found to induce oxidative stress and disrupt cell membrane integrity while their toxicity is reported to be size and dose dependent.³⁶⁻³⁸ BP is ranked as intermediately toxic in between TMDs and GO.³⁶ Arsenic (As) nanosheets were reported to have high toxicity as almost all cells were non-viable when exposed to high concentration of the material (200 µg mL⁻¹). However, antimony (Sb) and bismuth (Bi) nanosheets were found to have lower toxicities than GO, reduced GO (rGO) and BP.³⁹ Despite the vast reports on toxicity of layered materials, toxicities of layered MAX and MAB phases have yet to be studied and filling this research gap is imperative prior to any future research and commercial applications.

4.2 Results and Discussion

Material characterization

MAX and MAB phases were first characterized to evaluate the success of synthesis as well as understand how their physiochemical properties may correlate to their electrochemical performances and toxicity profiles. Their morphologies and thickness were examined by scanning electron (SEM), transmission electron (TEM) and atomic force (AFM) microscopy. Their crystal structures and phase purities were studied by X-Ray diffraction (XRD). Their elemental compositions and chemical bonding were observed by energy dispersive (EDS), X-Ray photoelectron (XPS) and Raman spectroscopy.

From SEM images obtained (**Figure 1A**), the materials were found to exist in the bulk state with apparent layered stacked arrangements as postulated.²⁰ However, two different morphological features were observed in which Ti_2AlN , Ti_3AlC_2 and Ti_3SiC_2 exhibited distinct sheet-like layers⁴⁰ while the layers in Ti_2AlC , Cr_2AlB_2 and MoAlB appeared rounded and clumpy. The bulk state is further confirmed by large thickness seen in TEM (**Figure 1B**) and AFM (**Figure S1**) images. Their thickness was found to range between 0.5 and 2 μm . Following that, EDS elemental maps (**Figure S2**) were captured to confirm the elemental distribution and compositions of the materials. The elemental maps obtained showed homogenous distributions of the respective elements on the surfaces of the samples. Their elemental compositions were calculated from atomic percentages obtained through EDS and the calculated elemental ratios were found to be relatively close to the stoichiometric ratios of each material (**Table S1**). The slight disparity from theoretical ratio can be attributed to the limited depth and area of analysis of this technique which may lead to a less precise quantification of elements present.

Figure 1. A) SEM and B) TEM images of respective powdered MAX and MAB phases.

In addition, XPS was conducted to study the surface chemical compositions of MAX and MAB phases. Wide survey XPS spectra (**Figure S3**) confirmed the presence of expected elements originating from the compound itself (i.e. Ti, Mo, Cr, Al, Si, C, B, N) as well as additional oxygen and carbon originating from air during materials handling prior to experiments.⁴⁰ High resolution scans were also obtained to examine the specific oxidation states of the elements; a limitation which wide survey scans possess. High resolution X-ray photoelectron spectroscopy (HR-XPS) spectra of transition metal (M) atoms, specifically Ti 2p, Mo 3d and Cr 2p core levels were obtained and presented in **Figure 2A**. The Ti 2p spectra obtained showed titanium oxide dominant peaks with lower intensity of titanium carbide and elemental Ti. This suggests that Ti from MAX phases can easily undergo oxidation forming titanium oxide.⁴¹ The dominant oxidation state in Mo 3d core level is Mo⁶⁺ with small amounts of Mo⁴⁺ and Mo⁰ while the dominant oxidation state from Cr 2p core level was found to be Cr³⁺ with some amounts of Cr⁰ and Cr⁴⁺. Since M-A bond is reported to be metallic,²⁰ it is expected that the A Group atoms

predominantly exist as Al and Si metals. However, they were found in forms of Al_2O_3 (Al^{3+}) and SiO_2 as presented in **Figure 2B** indicating the presence of oxidation. Following that, C, N and B 1s spectra showed presence of carbide, nitride and boride peaks as well as their oxidised counterparts (**Figure S4**). This significant degree of oxidation could have occurred during the handling of samples as a result of exposure to atmospheric oxygen. The respective peak assignments of transition metal (M), A Group atom (A) and X/B atom of MAX and MAB phases obtained from HR-XPS spectra are summarized in **Table S2**. However, XPS is a surface characterization method and has limited depth and area measurements. Hence, X-ray diffraction (XRD) patterns were studied and Raman spectroscopy was conducted to show more proof of successful synthesis.

Figure 2. High resolution XPS of **A)** metal (M) and **B)** A-atom (Al/Si) of respective MAX and MAB phases.

XRD patterns of the respective MAX and MAB phases (**Figure S5**) were acquired to determine their crystallographic structure and phase purity. The sharp narrow peaks observed confirm the high crystallinity of the materials. The diffraction patterns were in perfect match with each corresponding compound with some minor elemental impurities, such as carbon, titanium nitride or their oxidised species (marked with asterisks). The highest amount of impurities was observed in the XRD pattern of Ti_2AlC (PDF: 04-001-6560). The main impurity belonged to AlTi , with peaks arising at 2θ values of 21.97° , 31.82° , 38.92° , 44.58° , 45.54° , 65.53° , 66.27° , 78.10° , 79.50° and 83.19° . In addition, traces of titanium carbide (36.08° and 41.89°) and elemental carbon (27.09° and 41.05°) were also present. The amount of impurities was significantly fewer in XRD patterns of other MAX phases. The XRD pattern of Ti_2AlN (PDF: 00-055-0434) contained two additional peaks at 2θ of 36.80° and 42.71° due to presence of titanium nitride (TiN) while peaks due to titanium carbide (TiC) were observed at 2θ of 36.13° and 72.60° in the XRD pattern of Ti_3AlC_2 (PDF: 00-052-0875). Moreover, traces of aluminium oxide (Al_2O_3) were also found at 2θ of 25.69° , 43.46° and 57.60° . No additional peaks owing to impurities were detected from the XRD pattern of Ti_3SiC_2 (PDF: 04-004-0286). The peaks in the XRD spectra matched those of Cr_2AlB_2 (PDF: 01-072-1847) and MoAlB (PDF: 04-008-8908) respectively. However, there were small amounts of CrB and MoB impurities (marked with asterisks) detected in their respective XRD patterns.

Lastly, Raman spectroscopy was performed on the MAX and MAB phases (**Figure S6**). Ti_2AlC exhibited vibration modes at 269 and 361 cm^{-1} which correspond to $(\text{E}_{2g}(\Gamma_2) + \text{E}_{1g}(\Gamma_3))$ and $\text{A}_{1g}(\Gamma_4)$ respectively, while the Ti_2AlN spectrum revealed an intense peak at 154 cm^{-1} matching to the characteristic vibrational mode of $\text{E}_{2g}(\Gamma_1)$. $\text{E}_{2g}(\Gamma_1)$ vibrational mode was also obtained at 150 cm^{-1} for Ti_3AlC_2 and 154 cm^{-1} for Ti_3SiC_2 . In addition, both Ti_3AlC_2 and Ti_3SiC_2 displayed phonon modes of $\text{A}_{1g}(\Gamma_4)$ at 269 and 271 cm^{-1} , and $\text{A}_{1g}(\Gamma_6)$ at 610 cm^{-1} and 605 cm^{-1} .^{42,43} For

the MAB phases, Raman spectrum of Cr_2AlB_2 displayed peaks at 308, 344 and 550 cm^{-1} . To our knowledge, Raman spectra of chromium-based MAB phases have not been reported in literature. Nevertheless, the large sharp peak at 550 cm^{-1} is characteristic of the symmetric stretching of Cr-O-Cr for trivalent chromium of chromium oxide (Cr_2O_3)⁴⁴ which corresponds to the $A_{1g}(\Gamma_1)$ vibrational mode.⁴⁵ Hence, since the XPS spectrum revealed that the dominant oxidation state of chromium is Cr^{3+} and judging from the structure of the compound, it is believed that the peak corresponds to the stretching of Cr-B-Cr. Finally, MoAlB exhibited characteristic phonon modes at 200 and 254 cm^{-1} which correspond to $B_{3g}(\Gamma_1)$ and $A_{1g}(\Gamma_1)$ phonon modes respectively.⁴⁶

Having understood the chemical compositions, purities and morphologies of the synthesised MAB and MAX phases, we next set forth to explore the fundamental electrochemical and electrocatalytic properties of the materials. The intrinsic redox behaviour of the material upon application of an electrochemical potential,¹ also known as inherent electrochemistry, was first investigated. Inherent electrochemical properties were reported to limit the operating electrochemical potential window of materials.⁴⁷⁻⁴⁹ Cyclic voltammetry (CV) was performed with MAX/MAB-modified glassy carbon (GC) electrodes in phosphate buffer (PBS) electrolyte solution. The results in **Figure S7** suggested negligible inherent electroactivities as no additional anodic or cathodic peaks relative to unmodified GC were observed. Therefore, they do not possess limited potential windows for future electrochemical applications.

Subsequently, heterogeneous electron transfer (HET) rates were measured to study the suitability of MAX and MAB phases for electrochemical sensing applications. HET rates provide a preliminary representation on the efficiency of electron transfer between target analytes and the electrode surface.¹ Typically, a higher HET rate would imply lower overpotentials for electrochemical reactions. For this study, CV of ferro/ferricyanide ($[\text{Fe}(\text{CN})_6]^{4-/3-}$) redox probe with MAX/MAB-modified GC electrodes were performed. The results (**Figure 3A**) showed shifts in peak potentials and therefore altered the separation between the cathodic and anodic peak (peak-to-peak separation, ΔE_p). ΔE_p values were calculated (**Figure 3B**) and found to decrease for all materials except Ti_3AlC_2 . HET rate constants (k_{obs}^0) were subsequently calculated according to Nicholson's method⁴⁹ from ΔE_p values and summarized in **Table S3**. k_{obs}^0 represents the electron transfer capabilities of the respective materials for comparison and analysis. In general, these materials possessed higher

HET rates relative to unmodified GC ($k_{\text{obs}}^0 = 9.13 \times 10^{-5} \text{ cm s}^{-1}$) with the exception of Ti_3AlC_2 which had lower HET rate of $k_{\text{obs}}^0 = 6.38 \times 10^{-5} \text{ cm s}^{-1}$. MAB phases were found to have greater k_{obs}^0 values than MAX phases. MoAlB has the highest HET rate of $k_{\text{obs}}^0 = 1.65 \times 10^{-3} \text{ cm s}^{-1}$ followed by Cr_2AlB_2 with HET rate of $k_{\text{obs}}^0 = 7.33 \times 10^{-4} \text{ cm s}^{-1}$. Ti_3SiC_2 has the highest HET rate among the MAX phases with k_{obs}^0 value of $1.44 \times 10^{-4} \text{ cm s}^{-1}$. In addition, HET rates between MAX phases of the same stoichiometric ratios were compared. Comparing between Ti_2AlN and Ti_2AlC , substitution of C atom with N atom was found to decrease HET rate by 1.5 folds. Band structure and density of states studies reported that the basal plane of Ti_2AlC is more conductive than that of Ti_2AlN ⁵⁰ which may affect the respective HET rates. Comparing Ti_3AlC_2 and Ti_3SiC_2 , changing Al to Si atom enhanced HET rate by 3 times and this may be due to higher electrical conductivity of Ti_3SiC_2 as previously reported.⁵¹ Hence, MoAlB, Cr_2AlB_2 , Ti_3SiC_2 and Ti_2AlC are favourable materials for electrochemical sensing applications due to their high electron transfer efficiency. It is speculated that MAX and MAB phases have catalytically active basal planes. In a previous study, the catalytically active site of MoAlB was reported to be at its basal plane.⁵² In addition, MXenes, specifically Mo_2CT_x was reported to have catalytically active basal planes.^{53,54} MAX phases comprises of stacked sheets which makes up the layered structure. As their catalytically active sites are known to be on the basal planes, exfoliated MXenes would have higher catalytic performance as the exposed surface area of the available basal planes for catalytic activity is higher. The presence of impurities, such as CrB and MoB, could have an effect on the HET rates similar to the effect observed for other layered materials containing impurities such as transition metal dichalcogenides (TMDs with metallic impurities).⁵⁵

Figure 3. A) Cyclic voltammograms (vs. Ag/AgCl) of bare GC, MAX and MAB phases in $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe (10 mM) at a scan rate of 100 mV s^{-1} . **B)** Average peak-to-peak separations (ΔE_p) of GC and respective MAX/MAB phases with respective standard deviations from three measurements.

Having understood their electron transfer properties, we investigated their electrochemical performances towards the electrochemical detection of important biomarkers; ascorbic acid (**Figure 4A**) and uric acid (**Figure 4C**). CV scans with unmodified GC electrode resulted in anodic peaks centred $\sim +0.47 \text{ V}$ for both target analytes and the modified electrodes saw shifts in the peak positions. The anodic peaks obtained for both ascorbic and uric acid remained sharp and easily distinguishable due to the absence of inherent peaks for the respective materials (**Figure S7**). MoAlB, Cr₂AlB₂, Ti₂AlC and Ti₃AlC₂ showed improvements and selectivity towards the detection of ascorbic acid with a shift in their peak positions to lower potentials (0.386 V, 0.375 V, 0.380 V and 0.372 V respectively) as compared to GC (0.472 V) (**Figure 4B**). Similarly, MoAlB, Cr₂AlB₂, Ti₂AlC and Ti₃AlC₂ displayed slight improvement and selectivity in detection of uric acid with shifts to lower potentials (0.429 V, 0.426 V, 0.429 V and 0.447 V respectively) when compared to bare GC (0.478 V) (**Figure 4D**). The improved performances with MoAlB, Cr₂AlB₂ and Ti₂AlC can be attributed to higher HET rates which were previously calculated. The high k^0 values obtained for the above stated materials indicate

faster and more efficient electron transfer abilities. As such, the voltammetric peaks obtained shifted to lower peak potentials. Ti_3SiC_2 showed improvement in performance for uric acid detection but not ascorbic acid detection. Ti_2AlN was the least performing material for the detection of both target analytes with the smallest shift in peak potential obtained. This observation is predicted due to Ti_2AlN having HET rate almost identical to bare GC. Overall, with the exception of Ti_2AlN , MAX and MAB phases were able to enhance the performance of ascorbic and uric acid detection. The improvement in performance is, however, more significant for ascorbic acid detection than uric acid.

Figure 4. Cyclic voltammograms (vs. Ag/AgCl) of GC, MAX and MAB phases in **A)** ascorbic acid (5 mM) and **C)** uric acid (5 mM) at a scan rate of 100 mV s^{-1} . Average peak potentials of **B)** ascorbic acid and **D)** uric acid with respective standard deviations obtained from three measurements.

Electrocatalytic Performances

Next, their performances as electrocatalysts in energy applications specifically HER and ORR were studied. HER corresponds to the cathodic half reaction of electrolysis of water which produces hydrogen gas as fuel for energy production while ORR is an important cathode reaction during energy production in fuel cells technology.¹ HER and ORR typically require catalysts to efficiently drive the reactions forward. To date, platinum (Pt) serves to be the best and most efficient catalyst but due to low abundance, the use of Pt is not economically favourable. As such, the quest to discover alternative cheaper catalysts is essential. Hence, the performances of MAX and MAB phases as electrocatalysts were explored.

The electrocatalytic properties of MAX and MAB phases towards HER were studied by linear sweep voltammetry (LSV) (**Figure 5**). Unmodified GC and Pt serve as references to evaluate the electrocatalytic performances of the materials. Overpotential at current density of -10 mA cm^{-2} serves as performance indicators for HER efficiency which is commonly used in energy applications. Lower overpotential values would indicate better HER performance of a material. As predicted, Pt displayed superior HER electrocatalytic performance with the lowest overpotential value obtained (**Figure 5A**). From **Figure 5B**, most MAX and MAB phases had lower overpotentials than GC with the exception of Ti_2AlN . Both MoAlB and Ti_3SiC_2 displayed the lowest overpotential values of -0.74 V . Generally, MAX and MAB phases possess improved catalytic performance towards HER with MoAlB and Ti_3AlC_2 having the best performance amongst them. Nevertheless, the electrocatalytic performances were still significantly poorer compared to Pt.

Tafel slope calculations were next performed to provide insights into the predominant HER mechanism.⁵⁶ The respective Tafel slope value b can be obtained by plotting the linear range fitted to the Tafel equation of $\eta = a + b \log |j|$ (**Figure 5C**). Tafel slopes are determined by the rate limiting steps of HER involved as follows:⁵⁶⁻⁵⁸

1. Adsorption (Volmer process):

2. Desorption (Heyrovsky process):

Or Desorption (Tafel process):

HER mechanism first undergoes adsorption Volmer adsorption process to produce hydrogen atoms, followed by either a Heyrovsky or Tafel desorption step. Ti_3SiC_2 yielded the smallest Tafel slope value of $104.3 \text{ mV dec}^{-1}$ while Ti_2AlN yielded the highest Tafel slope value of $146.35 \text{ mV dec}^{-1}$. All of the MAX and MAB phases displayed Tafel slope values of $> 100 \text{ mV dec}^{-1}$ (**Figure 5D**). It can also be inferred that the limiting step for the MAX and MAB phases to be the Volmer adsorption process as their values are close to 120 mV dec^{-1} .

Figure 5. **A)** Linear sweep voltammograms (vs. RHE) of GC, Pt, MAX and MAB phases for HER in H₂SO₄ (0.5 M) electrolyte at a scan rate of 2 mV s⁻¹. **B)** Average overpotential of Pt, GC, MAX and MAB phases obtained at current density of -10 mA cm⁻² with their respective standard deviations from three measurements. **C)** Tafel plots and **D)** Tafel slopes values of MAX and MAB phases with their respective standard deviations from three measurements

Next, the electrocatalytic performances of MAX and MAB phases towards ORR were explored (**Figure 6A**). The LSV curves in ambient conditions showed distinct decrease in current which corresponds to the occurrence of ORR. The formation of a cathodic peak was observed for Pt centred at ~-0.28 V. The peak positions shifted in the presence of the MAX and MAB phases to ~-0.43 V. However, the cathodic peak for MoAlB did not appear in the same range. The respective cathodic peaks disappeared and were not apparent in N₂ purged conditions (**Figure 6B**). Their electrocatalytic ORR performances were evaluated and compared using their onset

potentials; the potential at which 10% of the maximum current is achieved (**Figure 6C**). With the exception of MoAlB, MAX and MAB phases possess much lower onset potentials than GC with Cr₂AlB₂ having the lowest onset potential of -0.17 V. The results obtained suggest that MAX and MAB phases are potential electrocatalysts for ORR with the exception of MoAlB. MoAlB appears to be a poor electrocatalyst for ORR owing to the high onset potential value obtained and diminished cathodic peak observed in its polarisation curve.

Figure 6. Linear sweep voltammograms (vs. Ag/AgCl) of GC, MAX and MAB phases for ORR in **A**) unpurged and **B**) purged conditions in KOH (0.1 M) at a scan rate of 50 mV s⁻¹. **C**) Average onset potentials of GC, MAX and MAB phases with respective $\pm$ standard deviations from three measurements.

In general, MAX and MAB phases have intermediate catalytic performances as compared to other reported layered materials which serve as electrocatalysts. MAX and MAB phases possess better HER catalytic performance than bulk pnictogen nanosheets¹² and Group 5 TMDs⁵⁹ with comparable performance to bulk Group 6 TMDs.⁷ However, it was noted that their performance could not match that of bulk platinum dichalcogenides⁸ and MXenes.⁶⁰⁻⁶² MXenes have been more widely reported as promising electrocatalyst supports for HER and ORR. Additionally, the presence of small amounts of catalytic metals in those materials displayed improvement in catalytic performance.^{63,64} For ORR, MAX and MAB phases possess higher catalytic performances than bulk pnictogen nanosheets¹² and comparable performances to bulk Group 6 TMDs⁷ but weaker than that of PtTe₂³⁴.

Cytotoxicity Assessments

In most studies, toxicity was preliminarily investigated by *in vitro* assessments using common mammalian cell lines such as human lung carcinoma cells (A549)³⁰⁻³⁶ as exposure of nanomaterials to the human body is highly likely through inhalation from the environment.^{65,66} Another commonly reported method of exposure includes ingestion and injection.^{65,66} With the expansion of nanomaterials into a myriad of applications, their risks to other parts of the body have to be also explored. In this study, *in vitro* cytotoxicity assessments were conducted on four different mammalian cell lines to investigate toxicity of MAX and MAB phases on different organs in the human body. The four mammalian cell lines used were human lung carcinoma cells (A549), human kidney cells (HEK 293), human breast carcinoma cells (MCF-7) and human liver carcinoma cells (HepG2). This serves as a preliminary study towards understanding the biocompatibility of MAX and MAB phases in the human body as well as an important evaluation towards their risks for future industrial and biological applications. To date, only few studies have reported on toxicity studies of these materials and their counterparts. For instance, Ti_3AlC_2 , Ti_3SiC_2 and Ti_2AlN were reported to be cytocompatible with Ti_2AlN exhibiting superior cell proliferation performance to a commercial Ti-based alloy and pure Ti.²⁴ Additionally, Ti_3AlC_2 is reported to be a safe and bioinert material which is commonly studied for biomaterials applications such as body implants.^{67,68}

Herein, cytotoxicity was assessed by measuring viability of cells after exposure to nanomaterials for 24 h using cell counting kit-8 (CCK-8); a sensitive colorimetric cell proliferation assay which utilizes a highly water-soluble tetrazolium salt (WST).⁶⁹ The tetrazolium salt which is present in CCK-8 undergoes reduction in the presence of dehydrogenase which is only available in viable cells and lead to the formation of soluble orange formazan dye that can be quantified by absorbance spectroscopy.⁶⁹ Hence, cell viability

can be quantified since the amount of formazan produced is directly proportional to the number of viable cells.

Prior to cytotoxicity assessments, control experiments were conducted to investigate the presence of interferences. Previously, it was reported that absorbance-based cell viability assays could produce false positive results due to absorbance from nanomaterials.^{35,36} The reported interferences include; 1) nanomaterials may reduce tetrazolium salt in CCK-8 to formazan in the absence of cells, and 2) nanomaterials may possess similar absorbance properties at the measured wavelength. With that, absorbance of nanomaterials, cell culture media and CCK-8 in the absence of cells was analysed prior to cytotoxicity assessments. The results of the control experiments are presented in **Figure S8**. The absorbance percentages of Ti_3AlC_2 , Ti_2AlN and Ti_2AlC were within the acceptable percentage range ($\pm 50\%$) at all concentrations, suggesting negligible interferences. However, absorbance percentages of Ti_3SiC_2 , Cr_2AlB_2 and MoAlB were above the acceptable percentage range. Absorbance percentage of Ti_3SiC_2 exceeded 150% at high concentration of $125 \mu\text{g mL}^{-1}$ while absorbance percentages of Cr_2AlB_2 and MoAlB exceeded the acceptable range at an even lower concentration of $25 \mu\text{g mL}^{-1}$. With that, absorbance spectra of Ti_3SiC_2 , Cr_2AlB_2 and MoAlB from 350 nm to 550 nm were obtained in the presence and absence of cells to elucidate the cause for the increase in absorbance (**Figure S9**). Peaks were observed at 450 nm for measurements in the presence of cells which corresponded to the absorbance of formazan dye (**Figure S9A, S9C and S9E**) while no peaks were observed at 450 nm for measurements in the absence of cells which suggested the absence of formazan dye (**Figures S9B, S9D and S9F**). As a result, this eliminates the possible interference induced due to the formation of formazan dye from the reduction of CCK-8 by nanomaterials. However, despite the absence of peaks, absorbance values increased with increasing concentration of nanomaterials. This would

suggest that the interferences observed were attributed to absorbance by nanomaterials themselves. Different nanomaterials possess different range and extent of absorbance. Hence, to obtain a more accurate representation of cell viability, the background absorbance of nanomaterials was deducted from the absorbance values obtained during cell viability studies. The cell viability results of MAX phases upon exposure to the various cell lines are presented in **Figure 7**. Cell viability of A549 (**Figure 7A**) remained relatively unchanged at nanomaterials concentrations between 0 and 125 $\mu\text{g mL}^{-1}$ which implies that MAX phases do not impose any cytotoxic effect on A549 cells. However, slight toxicity was observed when concentration of Ti_2AlN , Ti_3AlC_2 and Ti_2AlC increased to 150 $\mu\text{g mL}^{-1}$ as the cell viabilities decreased to 87%, 69% and 69% respectively. This suggests that Ti_3AlC_2 and Ti_2AlC are more toxic towards A549 cells followed by Ti_2AlN and Ti_3SiC_2 . Upon introduction of MAX phases to MCF-7 (**Figure 7B**), cell viability remained unchanged from 0 to 100 $\mu\text{g mL}^{-1}$. Thereafter, cell viability of all MAX phases continued to decrease as concentration of nanomaterials increases to 125 and 150 $\mu\text{g mL}^{-1}$. Exposure to 150 $\mu\text{g mL}^{-1}$ of Ti_3AlC_2 led to the largest decrease in cell viability to 54% whereas the other MAX phases dropped only to about 70% upon exposure at the same concentration. This suggests that Ti_3AlC_2 possesses the highest toxicity towards MCF-7. Cell viability of HEK 293 remained relatively high (> 80%) at all concentrations (**Figure 7C**) which suggest negligible toxicity of MAX and MAB phases towards HEK 293. When introduced to HepG2 (**Figure 7D**), cells which were exposed to 150 $\mu\text{g mL}^{-1}$ of Ti_3SiC_2 , Ti_2AlN and Ti_3AlC_2 showed significant decrease in cell viabilities to 65%, 70% and 78% respectively. This suggests that Ti_3SiC_2 , Ti_2AlN and Ti_3AlC_2 are more toxic towards HepG2 at higher concentrations with Ti_3SiC_2 being the most toxic. In general, MAX phases are most toxic towards MCF-7 as all materials displayed dose dependent toxicity above 100 $\mu\text{g mL}^{-1}$ of exposure. On the other hand, MAX phases may possibly display biocompatibility towards HEK 293 as negligible toxicity was observed at all tested

concentrations. Ti_3AlC_2 imposed the highest toxicity as its toxic effect is apparent towards 3 different cell lines; A549, MCF-7 and HepG2 cells. Nevertheless, toxic properties were only observable at high concentrations.

Figure 7. Percentage cell viability of A) A549 cells, B) MCF-7 cells, C) HEK 293 cells, and D) HepG2 cells upon introduction of varying concentrations of MAX phases (Ti_3SiC_2 , Ti_2AlN , Ti_3AlC_2 and Ti_2AlC) for 24 h. Cell viability at every concentration was normalized with readings of negative control ($0 \mu\text{g mL}^{-1}$) and averaged of three repeats with the respective $\pm$ standard deviations.

Cell viability results of A549, MCF-7, HEK 293 and HepG2 with MAB phases are presented in **Figure 8**. Cell viabilities across most tested concentrations of MAB remained above 80% which imply low toxicity on the cells. Nevertheless, higher concentrations of MAB caused observable differences in cell viability. Upon exposure of 125 and 150 $\mu\text{g mL}^{-1}$ to A549 (**Figure 8A**) and MCF-7 (**Figure 8B**) cells, cell viability reduced to below 80%. A sudden drop of cell viability to 39% was observed when 150 $\mu\text{g mL}^{-1}$ of MoAlB was exposed to MCF-7 cells. On the other hand, cytotoxicity of MAB when introduced to HEK 293 (**Figure 8C**) and HepG2 (**Figure 8D**) cells presented different trends. Cell viability of HEK 293 remained high (above 90%) upon exposure with 0 to 125 $\mu\text{g mL}^{-1}$ of Cr_2AlB_2 but decreased to 76% with 150 $\mu\text{g mL}^{-1}$ of Cr_2AlB_2 . Cell viability of HEK 293 when exposed to MoAlB only remained high (above 90%) at 0 to 75 $\mu\text{g mL}^{-1}$ of MoAlB. Subsequently, a dose-dependent toxicity trend is observed upon exposure of 100 $\mu\text{g mL}^{-1}$ (58%), 125 $\mu\text{g mL}^{-1}$ (44%) and 150 $\mu\text{g mL}^{-1}$ (31%) of MoAlB. Cell viability of HepG2 upon exposure to MAB remained relatively high (above 80%) across all concentrations. In general, cytotoxicity of MoAlB and Cr_2AlB_2 are similar towards all four cell lines. A difference in toxicity is only observed towards MCF-7 and HEK 293 cells in which MoAlB induced higher toxicity than Cr_2AlB_2 .

A comparison of the toxicities between MAX/MAB phases and other reported bulk layered materials (TMDs and phosphorus allotropes) was made by comparing cell viabilities after exposure to 25 $\mu\text{g mL}^{-1}$ of respective nanomaterials with A549 cells; the most commonly used cell line for cytotoxicity studies (**Table S4**). At this concentration, cell viability when introduced with MAX and MAB phases remained unchanged while the other materials showed apparent decreased in cell viabilities. This implies that MAX and MAB phases are the most non-toxic group of layered materials and are safe materials for human handling with potential biocompatibility in human for future biological applications.

Olszyna *et al.* recently reported toxicity studies of Ti_3C_2 MXene on a variety of cell lines including A549 cells.⁷⁰ Results from MTT and calcein-AM assays show decrease in cell viability and number of living cells with increasing concentration of Ti_3C_2 . Specifically, cell viability of A549 decreased to about 30% after exposure to 500 mg L^{-1} (i.e. 0.5 mg mL^{-1}) of Ti_3C_2 . As such, MAX phases (i.e. Ti_3SiC_2 and Ti_3AlC_2) have significantly lower toxicities than their etched counterpart, Ti_3C_2 .

Figure 8. Percentage cell viability of A) A549 cells, B) MCF-7 cells, C) HEK 293 cells, and D) HepG2 cells upon introduction of varying concentrations of MAB phases (Cr_2AlB_2 and MoAlB) for 24 h. Cell viability at every concentration was normalized with readings of negative control ($0 \text{ } \mu\text{g mL}^{-1}$) and averaged of three repeats with the respective $\pm$ standard deviations.

4.3 Conclusion

In summary, six different MAX and MAB phases (Ti_2AlC , Ti_2AlN , Ti_3AlC_2 , Ti_3SiC_2 , Cr_2AlB_2 , and MoAlB) were successfully synthesized and characterized. Following that, their electrochemical properties and electrocatalytic applications were explored. From the results, these materials possess negligible inherent electroactivities and displayed no apparent electrocatalytic properties towards OER. Interestingly, with an exception from Ti_2AlN , the MAX and MAB phases possess high HET rates, improved performance for ascorbic acid and uric acid electrochemical detection and displayed enhanced electrocatalytic performances towards HER and ORR. In overall, MAX and MAB phases have intermediate electrocatalytic performances as compared to other recently reported layered catalysts. In addition, the materials have near negligible toxicities towards lung, kidney, liver and breast cancer cells. Despite their dose dependent toxicity, they are found to be the most non-toxic group of layered materials thus far reported. Hence, at low concentrations, Ti_2AlC , Ti_3AlC_2 , Ti_3SiC_2 , Cr_2AlB_2 , and MoAlB are safe and promising materials for future sensing and energy applications with potential biocompatibility in human for future biological applications.

4.4 Experimental Section

Instruments

SEM images and EDS elemental mappings were captured by JEOL 7600F (Jeol, Japan). XPS measurements were conducted with Phoibos 100 spectrometer by a monochromatic Mg X-ray radiation source (SPECS, Germany) and analysed by CasaXPS software. XRD patterns were obtained using a Bruker D8 diffractometer in Bragg–Brentano para-focusing geometry with application of CuK α radiation. Diffraction patterns between 5° and 90° of 2 θ were collected. HighScore Plus 3.0e and Topas 5.0 software were used to evaluate the obtained data. InVia Raman microscope (Renishaw, England) in backscattering geometry with CCD detector was used for Raman spectroscopy. DPSS laser (532 nm, 50 mW) with applied power of 10 mW and 50 times magnification objective was used for the measurement. Electrochemical measurements were conducted using a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) and analysed by NOVA version 1.10 software. Absorbance readings were measured by Thermo Multiskan GO and analysed by SkanIt software for microplate readers (Thermo Fisher Scientific, Singapore).

Synthesis of MAX and MAB phases

Six MAX and MAB phases – Ti₂AlC, Ti₂AlN, Ti₃AlC₂, Ti₃SiC₂, Cr₂AlB₂, and MoAlB - were synthesized separately using different precursors, but following the same experimental procedure based on molten salts as reported previously.¹ Ti, Al (-325 mesh, Alfa Aesar) and C (mean particle size 2 μ m, Alfa Aesar) powders were mixed in stoichiometric quantities to synthesize Ti₂AlC and Ti₃AlC₂; Ti, Al and TiN (-325 mesh, Alfa Aesar) powders were used for Ti₂AlN, meanwhile Ti, Si (-325 mesh, Alfa Aesar) and C powders were the precursors for Ti₃SiC₂. Elemental powders of Cr (-325 mesh, Alfa Aesar), Al and CrB₂ (mean particle size 2.5 μ m, H. C. Stark) were stoichiometrically mixed to synthesize Cr₂AlB₂, and Mo (-325 mesh,

Alfa Aesar), Al, and AlB_2 (mean particle size 10 μm , Rboschco) powders were used to synthesize MoAlB . Subsequently, KBr in a weight ratio of 1:1 was added to each mixture of powders, and mixed for 24 h in a multidirectional mixer (Turbula, WAB) using 5 mm zirconia balls and ethanol as liquid media. Slurries were dried in a rotary evaporator, and the dried powders were sieved through 300 μm to homogenize. The powder was uniaxially pressed at 200 MPa, followed by isostatic pressing at 300 MPa. The different pellets were separately placed in alumina crucibles, which were filled with KBr salt. The samples were heated in an open furnace (Nabertherm, Germany) at a rate of 20 $^{\circ}\text{C min}^{-1}$ up to 800 $^{\circ}\text{C}$, followed by a rate of 5 $^{\circ}\text{C min}^{-1}$ up to the maximal temperature for 1 h. Maximal temperatures were different for each MAX phases, at 1000 $^{\circ}\text{C}$ for Ti_2AlC , 1250 $^{\circ}\text{C}$ for Ti_2AlN , Ti_3SiC_2 , Cr_2AlB_2 , MoAlB , and 1300 $^{\circ}\text{C}$ for Ti_3AlC_2 . The synthesized MAX phase powders were then washed repeatedly with hot distilled water to eliminate the salt, and dried overnight at 90 $^{\circ}\text{C}$.

Electrochemical measurements of MAX and MAB phases

MAX and MAB phases dispersed in ultrapure water (5 mg mL^{-1}) were subjected to ultrasonication for 1 h to obtain well-dispersed suspensions. Prior electrochemical measurements, the suspensions were subjected to ultrasonication for another 10 minutes to maintain well-dispersed samples. 3 μL of suspensions were drop-casted onto bare GC electrodes and left to dry under a lamp. Inherent electrochemistry studies were carried out by cyclic voltammetry at a scan rate of 100 mV s^{-1} in 50 mM phosphate-buffered saline (PBS, pH 7.0) solution. Heterogeneous electron transfer (HET) rates were obtained by cyclic voltammetry at a scan rate of 100 mV s^{-1} in 10 mM ferro/ferricyanide $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe. Electrochemical sensing of ascorbic acid and uric acid were conducted by cyclic voltammetry at a scan rate of 100 mV s^{-1} . Hydrogen evolution reaction (HER) was investigated by linear sweep voltammetry at a scan rate of 2 mV s^{-1} in 0.5 M H_2SO_4 . Oxygen reduction

reaction (ORR) was studied by linear sweep voltammetry in both purged and ambient conditions at a scan rate of 50 mV s^{-1} with 0.1 M KOH as electrolyte. All measurements were conducted relative to Ag/AgCl reference electrode unless otherwise stated and Pt electrode as counter electrode.

Cytotoxicity assessments

A549 (Bio-Rev, Singapore), MCF-7, HepG2 and HEK-293 cells (ATCC, Manassas, VA, USA) were cultured by incubating at 37°C with $5\% \text{ CO}_2$ in cell culture media (CCM) that was prepared from Dulbecco's modified eagle medium (DMEM), 10% fetal bovine serum (FBS) (Life Technologies, Singapore) and 1% penicillin/streptomycin (Capricorn). Thereafter, 3000 cells per well were seeded in a 96-well plate and incubated under similar conditions for 24 h. MAX and MAB phases were dispersed in ultrapure water to a concentration of 0.5 mg mL^{-1} . The suspensions were subjected to ultrasonication of 1 h to obtain well-dispersed suspensions. Prior to each cytotoxicity assessment, the suspensions were ultrasonicated for another 10 min to maintain well-dispersed samples. After 24 h incubation of the seeded cells, the CCM were removed and replaced with varying concentrations ($0 - 150 \text{ }\mu\text{g mL}^{-1}$) of MAX and MAB phases suspensions which were previously prepared in fresh CCM. Cells with CCM only were used as negative control and CCM with nanomaterials only was used as control. Subsequently, the cells and nanomaterials dispersion ($100 \text{ }\mu\text{L}$ per well) were incubated at 37°C under $5\% \text{ CO}_2$ for another 24 h. Following that, cell viabilities were quantified by water-soluble tetrazolium (WST-8) assay (Dojindo, Japan). $10 \text{ }\mu\text{L}$ of WST-8 reagent was added per well and further incubated for 1 h. After 1 h, absorbance was measured at 450 nm and absorbance spectrum from 350 nm to 550 nm was obtained.

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4.5 Supporting Information

Figure S1. AFM images of respective MAX and MAB phases.

Figure S2. EDS images and corresponding elemental maps of respective MAX and MAB phases. Scale bar represents 1 μm .

Table S1. Atomic percentages of elements of MAX and MAB phases from EDS. Ratios of metal to A Group atom (M/A), and X atom to A Group atom (X/A) were calculated.

Compound	M (Ti, Cr, Mo)	A (Al, Si)	X (C, N) or B	M/A	X/A
Ti ₂ AlC	46.87	19.44	31.47	2.4	1.6
Ti ₂ AlN	41.02	15.66	14.17	2.6	0.9
Ti ₃ AlC ₂	36.87	10.88	29.22	3.4	2.7
Ti ₃ SiC ₂	41.07	11.83	28.74	3.5	2.4
Cr ₂ AlB ₂	41.37	26.80	31.83	1.5	1.2
MoAlB	23.09	27.55	49.36	0.47	1.8

Figure S3. XPS wide survey spectra of respective MAX and MAB phases.

Figure S4. High resolution XPS 1s spectra of X (C, N) or B atoms of respective MAX and MAB phases.

Table S2. Summary of peak assignments of elements obtained from HR-XPS.

Material	Element	Peak Assignments	Region	Binding Energy (eV)
Ti₂AlC	Ti	Ti-C	Ti 2p _{3/2}	454.75
			Ti 2p _{1/2}	460.75
		Ti-O	Ti 2p _{3/2}	458.07
			Ti 2p _{1/2}	464.07
	Al	Al (III)	Al 2p _{3/2}	74.25
			Al 2p _{1/2}	74.69
		Al	Al 2p _{3/2}	70.99
			Al 2p _{1/2}	71.43
	C	Adventitious	C 1s	284.53
		C-C	C 1s	285.21
		Ti-C	C 1s	281.40
		C=O	C 1s	288.50
Ti₃AlC₂	Ti	Ti-C	Ti 2p _{3/2}	454.76
			Ti 2p _{1/2}	460.76
		Ti-O	Ti 2p _{3/2}	457.93
			Ti 2p _{1/2}	463.63
		Ti	Ti 2p _{3/2}	453.41
			Ti 2p _{1/2}	459.41
	Al	Al (III)	Al 2p _{3/2}	74.19
			Al 2p _{1/2}	74.63
		Al	Al 2p _{3/2}	70.91
			Al 2p _{1/2}	71.35
	C	Adventitious	C 1s	284.69
		C-C	C 1s	285.17
		Ti-C	C 1s	281.07
		C=O	C 1s	288.85
Ti₃SiC₂	Ti	Ti-C	Ti 2p _{3/2}	455.31
			Ti 2p _{1/2}	461.41
		Ti-O	Ti 2p _{3/2}	458.27
			Ti 2p _{1/2}	463.97
		Ti	Ti 2p _{3/2}	453.76
			Ti 2p _{1/2}	459.46
	Si	SiO ₂	Si 2p _{3/2}	101.92
			Si 2p _{1/2}	102.55
		Si	Si 2p _{3/2}	98.64
			Si 2p _{1/2}	99.27
Ti₂AlN	Ti	Adventitious	C 1s	284.79
		Ti-C	C 1s	281.04
Ti₂AlN	Ti	Ti-N	Ti 2p _{3/2}	455.97

		Ti-O	Ti 2p _{1/2}	461.69
			Ti 2p _{3/2}	457.77
			Ti 2p _{1/2}	463.49
		Ti	Ti 2p _{3/2}	453.45
			Ti 2p _{1/2}	459.17
	Al	Al (III)	Al 2p _{3/2}	74.04
			Al 2p _{1/2}	74.48
		Al	Al 2p _{3/2}	70.71
			Al 2p _{1/2}	71.15
	N	Ti-N	N 1s	395.87
		N-O	N 1s	399.08
MoAlB	Mo	Mo (VI)	Mo 3d _{5/2}	232.85
			Mo 3d _{3/2}	236.00
		Mo (IV)	Mo 3d _{5/2}	231.15
			Mo 3d _{3/2}	234.30
		Mo	Mo 3d _{5/2}	227.90
			Mo 3d _{3/2}	236.00
	Al	Al (III)	Al 2p _{3/2}	74.65
			Al 2p _{1/2}	75.09
		Al	Al 2p _{3/2}	71.98
			Al 2p _{1/2}	72.42
	B	B ₂ O ₃	B 1s	192.63
		Boride	B 1s	188.31
Cr₂AlB₂	Cr	Cr (VI)	Cr 2p _{3/2}	580.44
			Cr 2p _{1/2}	589.74
		Cr (III)	Cr 2p _{3/2}	576.20
			Cr 2p _{1/2}	585.50
		Cr	Cr 2p _{3/2}	573.21
			Cr 2p _{1/2}	582.51
	Al	Al (III)	Al 2p _{3/2}	74.25
			Al 2p _{1/2}	74.69
		Al	Al 2p _{3/2}	71.32
			Al 2p _{1/2}	71.79

	B	B ₂ O ₃	B 1s	191.45
		B	B 1s	187.13

Figure S5. X-ray diffraction patterns of respective MAX and MAB phases.

Figure S6. Raman spectra of respective MAX and MAB phases.

Figure S7. Cyclic voltammograms (vs. Ag/AgCl) of bare GC, MAX and MAB phases in PBS (50 mM, pH 7.0) electrolyte at a scan rate of 100 mV s⁻¹.

Table S3. Summary of peak-to-peak separations (ΔE) and HET rate constants (k_{obs}^0) of GC, MAX and MAB phases.

	ΔE (mV)	k_{obs}^0 (cm s ⁻¹)
GC	383.30	9.13×10^{-5}
MoAlB	169.91	1.65×10^{-3}
Cr₂AlB₂	229.88	7.33×10^{-4}
Ti₂AlC	349.81	1.44×10^{-4}
Ti₃AlC₂	409.77	6.38×10^{-5}
Ti₃SiC₂	319.82	2.16×10^{-4}
Ti₂AlN	379.79	9.58×10^{-5}

Figure S8. Percentage absorbance of MAX and MAB phases in cell-free conditions in the presence of cell culture media and CCK-8 measured at wavelength of 450 nm. The absorbance readings are normalized to the control set and are a mean of triplicates with their respective standard deviations.

Figure S9. Absorbance spectra of **A)** MoAlB, **C)** Cr₂AlB₂ and **E)** Ti₃SiC₂ in the presence of cells and **B)** MoAlB, **D)** Cr₂AlB₂ and **F)** Ti₃SiC₂ in the absence of cells. All measurements were carried out in cell culture media and CCK-8 in concentration range of 0 – 150 µg mL⁻¹ of nanomaterials.

Table S4. Comparison of normalized cell viability percentages of A549 cells by WST-8 assay after 24 h exposure of 25 $\mu\text{g mL}^{-1}$ of layered nanomaterials in their bulk forms.

Nanomaterial	Cell Viability (%)	Reference
MoAlB	100	This work
Cr ₂ AlB ₂	100	This work
Ti ₂ AlC	100	This work
Ti ₃ AlC ₂	100	This work
Ti ₃ SiC ₂	100	This work
Ti ₂ AlN	100	This work
VTe ₂	50	48
NbTe ₂	90	48
TaTe ₂	100	48
PtS ₂	100	13
PtSe ₂	85	13
PtTe ₂	45	13
GaSe	87	50
GeS	55	50
BP	81	51
Black Phosphorus (Vapour Phase Growth)	45	54
Black Phosphorus (High Pressure Conversion)	60	54
Violet Phosphorus	70	54
Red Phosphorus	82	54

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Chapter 5

Electrochemical Applications and Cytotoxicity of
Functionalized Group 14 Layered Materials:
Siloxene, Germanane, and Methylgermanane

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5.1 Introduction

The ground-breaking research on graphene¹ has ignited intense interest in various two-dimensional (2D) elemental nanomaterials.²⁻⁹ These materials include group 14 mono-elemental graphene analogues known as silicene and germanene. Similar to graphene, they are 2D materials arranged in a honeycomb structure,¹⁰ but unlike planar graphene, they have buckled sheets.^{11,12} They exhibit similar promising electronic properties to graphene including low effective masses and high carrier mobilities but have a similarly negligible band gap, which restricts their applications in electronics.¹³ In addition, silicene and germanene are mainly synthesized on substrates due to lack of thermodynamic stability.^{2,14} Hence, various modifications of silicene and germanene have been carried out to improve their stability and increase their band gaps. Several modified silicene and germanene analogues were reported, e.g., siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$), germanane (Ge_6H_6) or methylgermanane ($\text{Ge}_6(\text{CH}_3)_6$) (**Scheme 1**).

Scheme 1. Chemical structure of siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$), germanane (Ge_6H_6) and methylgermanane ($\text{Ge}_6(\text{CH}_3)_6$).

Siloxene, germanane and methylgermanane can be prepared by topochemical deintercalation of their Zintl phases. Siloxene is -H and -OH modified silicene, prepared by topochemical deintercalation of Zintl phase CaSi_2 .¹⁵⁻¹⁸ CaSi_2 possess interconnected Si_6 rings in puckered arrangements held together by Ca^{2+} cations in a planar layered structure. These Ca^{2+} interlayers can be removed without disrupting the Si sheets, leaving each Si atom terminated by either -H

or -OH. Siloxene is reported to be semiconducting and has a direct band gap.¹⁹ Meanwhile, germanane which is essentially hydrogen-terminated germanene is typically prepared by topochemical deintercalation of CaGe_2 .²⁰ The hydrogenation termination of germanene results in the opening of band gap which alters its electronic properties.²¹ Germanane is semiconducting, possesses a direct band gap with strong infrared photoluminescence.²² Other unique properties of germanane include thermal stability and high resistance towards oxidation.²² On the other hand, methyl-terminated germanene, methylgermanane, was first synthesized by Jiang et al.²³ and found to be semiconducting and have a direct band gap of 1.76 eV.²⁴ Some interesting properties of methylgermanane includes strong photoluminescence, band edge fluorescence as well as improved thermal stability.²³ Methylgermanane is also reported with higher resistance against oxidation than germanane.²³

Due to their unique and promising properties, these materials were extensively explored for several applications. Siloxene has been reported as a promising photocatalyst for efficient water-splitting reactions,¹⁹ an anode material in Na^+ , Li^+ and K^+ ion batteries,²⁵ and a novel material for electrodes used for high-performance supercapacitor.²⁶ Germanane is reported to exhibit photocatalytic properties towards hydrogen generation through water splitting and hydrolysis of ammonia-borane complex.^{27,28} Methylgermanane has been reported as a fluorescence marker on nanographene-platinum microrobots and photocatalysts for hydrogen generation.²⁷⁻³⁰

To the best of our knowledge, electrochemical applications of siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$), germanane (Ge_6H_6) and methylgermanane (Ge_6Me_6) remain unexplored. In this study, we focus on investigating their electrochemical detection and catalyzation properties specifically

for detection of an important biomarker, dopamine, as well as the catalysis of important energy reactions, which includes the oxygen reduction and hydrogen evolution reactions. In addition, we further examined their toxic properties to understand the safety risks they may pose to the human health. Prior to any electroanalysis studies, the materials were characterized to deeper comprehend their respective properties.

5.2 Results and Discussion

Materials Characterization

Scanning electron microscopy (SEM) in **Figure 1A-1C** show all materials with structural features typical of their bulk forms. Siloxene (**Figure 1A**) and methylgermanane (**Figure 1C**) appear to have sheet-like morphologies,^{26,30} while germanane (**Figure 1B**) appears to have a layered block-like morphology with more angular edges.²⁷ Elemental maps obtained from energy dispersive spectroscopy (EDS) show homogeneous distribution of Si and O for siloxene (**Figure S1A**), Ge for germanane (**Figure S1B**) and Ge and C for methylgermanane (**Figure S1C**). Oxygen is observed on germanane and methylgermanane indicating possible oxidation of materials whereas carbon is observed only on germanane attributing to carbon from carbon tape and adventitious carbon from the environment. In addition, atomic force microscopy (AFM) was conducted to study the thickness and size of the materials (**Figure S2**). The results obtained show that siloxene have width ranging between 2 – 3 μm while its thickness varies from about 100 to 800 nm. Similarly, methylgermanane possess width ranging from 2 to 3.5 μm but with thickness of only 100 to 300 nm. Germanane have the largest size as its width was measured as 4 μm and with thickness of about 200 nm.

Figure 1. SEM images of **A)** siloxene, **B)** germanane and **C)** methylgermanane.

The materials were then characterized by X-ray powder diffraction (XRD) and high-resolution transmission electron microscopy (HR-TEM). From the XRD analyses (**Figure 2**), the diffuse peaks suggest that the materials are more amorphous than crystalline, which is in accordance with 2D nature of these materials. From the XRD, the interlayer distance between two methylgermanane (GeMe) sheets is 8.9Å (2θ 10.0°). Germanane (GeH), which contains less bulky hydrogen instead of methyl groups, has interlayer distance 5.7Å (2θ 15.6°). For siloxene, the interlayer distance is about 6.4Å (2θ 13.8°). These amorphous structures were also confirmed by HR-TEM (**Figure S3**). All materials contain traces of residual germanium or silicon (labelled by star in the **Figure 2**), respectively.

Figure 2. XRD spectra of methylgermanane, germanane and siloxene. Star labelled peaks correspond to residual germanium or silicon, respectively.

Next, X-ray photoelectron spectroscopy (XPS) was employed to further confirm the elemental compositions of the materials. The XPS survey scans in **Figure S4A – S4C** show Si, Ge, C and O peaks as anticipated. C and O peaks are seen due to surface functionalization, oxidation of materials or exposure to adventitious C and O. In addition, Cl peaks are found in siloxene and germanane while an iodine peak is observed in methylgermanane as methyl iodide was involved in preparation of materials. These impurities have been also previously reported in other studies.^{20,23} High-resolution XPS (HR-XPS) was conducted to deeper analyze the success of modification and the corresponding chemical environments of the materials. The modification of silicene to siloxene leads to the formation of -H and -OH bonds on Si.¹⁶ These bonds can be confirmed from HR-XPS Si 2p and O 1s scans. The Si 2p scan (**Figure 3A**) shows two peaks centered at 102.4 eV and 99.8 eV, which are attributed to Si-O and Si-Si/Si-H peaks, respectively.^{19,26,31} An O 1s scan of siloxene (**Figure S4D**) shows two peaks centered at 531 eV and 529.8 eV attributed to Si-O and adventitious oxygen (e.g. intercalated water) peaks, respectively.^{26,31} Adventitious oxygen is present due to exposure of samples to the atmosphere. The modification of calcium germanide to germanane and methylgermanane resulted in the formation of Ge-H and Ge-C bonds respectively. From HR-XPS Ge 3d scans of germanane (**Figure 3B**) and methylgermanane (**Figure 3C**), two peaks centered at 29.8 eV and 31.1 eV are observed and attributed to Ge-H and Ge-C respectively.^{27,28} The successful formation of -H and -C bonds on Ge are confirmed by peak shifts to higher binding energies relative to Ge metal.³² Ge-C resulted in a larger shift in binding energy as compared to Ge-H as electronegativity of C is higher than H.^{33,34} In addition, C 1s peak belonging to Ge-C was observed at 282.3 eV confirming the bonding of C on Ge.³⁰ Other peaks obtained from HR-XPS of O 1s and C 1s (**Figure S4E – S4I**) are attributed to adventitious C and O due to the exposure of materials to the atmosphere.

Figure 3. HR-XPS scans of (A) Si 2p from siloxene, (B) Ge 3d from germanane and (C) Ge 3d from methylgermanane.

The materials were then characterized by Fourier transformed infrared (FTIR) spectroscopy. The FTIR spectrum of siloxene (**Figure S5A**) shows distinct peaks corresponding to Si-OH and Si-H at 1008 and 2104 cm^{-1} .^{25,26,31,35} FTIR spectra of germanane (**Figure S5C**) and methylgermanane (**Figure S5E**) both show absorption bands corresponding to H_2O (1635 cm^{-1}). In addition, methylgermanane possess absorption bands for Ge-C (530 cm^{-1}) and CH_3 at 769 , 1236 and 2906 cm^{-1} while germanane have Ge-H bands at 472 cm^{-1} and 1998 cm^{-1} .^{20,22,23,36} Following that, the crystallinity and bonding nature of the materials were studied by Raman spectroscopy. **Figure S5B** shows the Raman spectrum of siloxene with one main band at 497 cm^{-1} corresponding to Si-O vibrations. Several weaker bands are also observed at 380 cm^{-1} consistent with Si-Si vibrations as well as 636 , 732 and 2119 cm^{-1} attributed to Si-H vibrations.^{19-26,31} Raman spectra of germanane (**Figure S5D**) and methylgermanane (**Figure S5F**) have an observable peak at $\sim 300\text{ cm}^{-1}$ consistent with the Ge-Ge vibration E_2 mode.^{22,36} These characterization results are in close agreement to our previous work which implies successful synthesis of materials.³⁷

As the experiments were performed in the aqueous conditions, stability of these materials could be an issue. Therefore, stability test of these materials we performed by measuring FTIR and

Raman spectra before and after 1 week in water. According to the spectra, there is no obvious change in material composition (**Figure S6 and S7**). FTIR spectra were measured on diamond ATR, therefore, the scale between 1950 and 2200 cm^{-1} is not displayed.

Fundamental Electrochemical Studies

Understanding fundamental electrochemical properties of materials is important for their prospective applications. Inherent electrochemical properties of siloxene, germanane and methylgermanane were explored to understand their intrinsic redox behaviour upon application of an electrochemical potential. To study this, glassy carbon (GC) electrodes were modified by these materials respectively and cyclic voltammetry was performed over a potential range of -1.5 to +1.5 V in phosphate buffered saline (PBS) electrolyte as presented in **Figure 4A**. From the results, an irreversible oxidation peak is observed at +0.7 V for methylgermanane while the others have negligible redox peaks. This implies that oxidation of Ge⁺ to higher oxidation states, possibly Ge^{2+/3+}, have occurred. One possible reason is that a few Ge atoms are still exposed at the surface of the material as the bulky methyl groups are not fully covering the Ge layers, resulting in the occurrence of oxidation.³⁸ Since inherent properties may limit the operating potential window of materials, the absence of peaks in siloxene and germanane allows more opportunities for prospective electrochemical applications.^{39,40}

Next, we set forth to study the rate of heterogeneous electron transfer (HET), an important factor for assessing the compatibility of material for electrochemical applications. A fast HET rate suggests fast kinetics and is therefore a desirable attribute of a prospective electrode material. To study HET, cyclic voltammetry of [Fe(CN)₆]^{3-/4-} redox probe in KCl (0.1 M) with material modified electrodes were studied. The results in Figure 4B showed shifts in cathodic and anodic peaks which brought about a change in peak-to-peak separation (ΔE_p). The ΔE_p values are presented in **Figure 4C**. Germanane and methylgermanane have lower ΔE_p values of 155 and 138 mV respectively, while siloxene has a higher ΔE_p of 248 mV which suggest HET rates for germanium materials are relatively higher. Nevertheless, all three materials possess smaller ΔE_p than GC which implies that modification of electrode with these materials

can successfully improve HET rates. To confirm this, HET rate constants (k_{obs}^0) were then obtained from ΔE_p values which are presented in **Table S1**. The calculated k_{obs}^0 of siloxene is 5 times higher than that of GC, while the calculated k_{obs}^0 of germanane and methylgermanane, 2.01×10^{-3} and $2.57 \times 10^{-3} \text{ cm s}^{-1}$ respectively, are approximately 17 and 21 times higher than the k_{obs}^0 of GC ($1.18 \times 10^{-4} \text{ cm s}^{-1}$). In a nutshell, germanane and methylgermanane have high HET rates (compared to GC) which is a favourable property for electrode materials in electrochemical applications.

Figure 4. Cyclic voltammograms (CVs) of GC, siloxene, germanane and methylgermanane in (A) PBS (50 mM), (B) $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe (10 mM) in KCl (0.1 M) and (C) peak-to-peak separations (ΔE_p) with their corresponding standard deviation. All measurements are performed using Pt counter electrode at scan rate 100 mV s^{-1} vs Ag/AgCl reference electrode.

Electrochemical Detection

Generally, due to high HET rates, two-dimensional (2D) materials can be integrated on electrode surfaces to increase selectivity and sensitivity of electrochemical sensors to target molecules.^{41,42} Properties such as large surface area, enhanced mass transport, excellent signal-to-noise ratios and high sensitivity have resulted in the vast incorporation of 2D materials in sensing applications.⁴³ Dopamine (DA), is a vital neurotransmitter in the brain for optimal performance of the central nervous system, cardiovascular system and hormonal system. DA in abnormal levels has been known to lead to conditions such as Parkinson's disease, schizophrenia, attention deficit hyperactivity disorder and restless legs syndrome (RLS).⁴⁴ However, detection of DA is challenging owing to its low physiological concentration along with interferences from other biomolecules, specifically ascorbic acid (AA) and uric acid (UA), due to overlapping oxidation potentials.^{45,46} Since then, novel 2D materials have been explored to enhance selectivity and sensitivity of DA detection.⁴⁵⁻⁵¹ Commonly reported materials are carbon-based and gold-based materials.

As DA is an electroactive species, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed to evaluate the performance of siloxene, germanane and methylgermanane modified electrodes for selective detection of DA. The redox reaction of DA involves two electrons transfer to form dopamine o-quinone and this two electrons transfer can be detected electrochemically.⁵² Since DA commonly coexists with AA and UA in biological samples, a selective detection is highly desired. CV was performed with the modified electrodes in a solution mixture containing AA (1.0 mM), UA (0.1 mM) and DA (0.1 mM). These respective concentrations are chosen as they fall within their commonly reported linear range and the linear range of AA is generally higher than that of UA and DA.⁴⁶ From **Figure**

5A, two distinct peaks are seen from the germanane modified electrode (green line) at +0.42 V and +0.61 V. Similarly, the methylgermanane modified electrode (purple line) show distinct separation of peaks at +0.38 V and +0.65 V. However, peak currents from methylgermanane are significantly lower than that of germanane. This suggests that methylgermanane possesses selectivity but lacks sensitivity. Bare GC (blue line) and siloxene modified electrode (red line) both have negligible peak separations which eliminates siloxene as a material for selective detection of DA.

After establishing germanane as the most promising material with high selectivity and sensitivity, the identities of the peaks were investigated. In **Figure 5B**, germanane was utilized to detect AA, UA and DA simultaneously and individually as a comparison. The peak potentials of AA and UA are observed to be close to each other specifically, at +0.31 V (red line) and +0.40 V (blue line) respectively. As the peaks are close together, simultaneous detection of the UA and AA result in an overall broad peak centered at +0.42 V as seen in the simultaneous scan (black line). However, the peak potential of DA is found at a higher potential of +0.64 V (green line) and is reflected as a separate peak in the simultaneous scan (black line). This shows that the germanane modified electrode can separately detect DA from AA and UA due to its higher peak potential. Hence, germanane can aid in the selective detection of DA with good sensitivity.

Figure 5. (A) Cyclic voltammograms (CVs) obtained with GC, siloxene, germanane and methylgermanane modified electrodes with simultaneous addition of AA (1.0 mM), UA (0.1 mM) and DA (0.1 mM). (B) CVs obtained with germanane modified electrode with simultaneous addition of AA, UA and DA (black line), AA only (red line), UA only (blue line) and DA only (green line). These measurements were performed in PBS (50 mM) using Pt counter electrode at a scan rate of 100 mV s^{-1} vs Ag/AgCl reference electrode.

Following that, DPV was used for calibration studies of DA. In **Figure S8A**, an increase in peak currents with increasing concentration of DA is observed. The respective current peaks are obtained and plotted against the concentration of DA (**Figure S8B**). In the range of 30 μM to 100 μM of DA, a linear relationship was obtained with a correlation coefficient of $R^2 = 0.988$. The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 19.1 μM and 63.9 μM respectively. To calculate sensitivity, the electroactive surface area is foremostly required. CVs were performed for the germanane modified electrodes in ferroferri at different scan rates (**Figure S9A**). The measured anodic current is plotted against square root of scan rate, showcasing a linear relationship which implies a diffusion controlled process (**Figure S9B**). At the same time, the gradient obtained from Randles Sevcik equation, $i_p = 0.446nFAC^0(\frac{nF_vD_0}{RT})^{1/2}$, was used to compute the electroactive surface area, A . Sensitivity is then obtained to be 0.308 $\mu\text{A}\mu\text{M}^{-1}\text{cm}^{-2}$. Stability of germanane modified electrodes after 1, 3 and 6 days were investigated by monitoring the changes to DA peak obtained from DPV (**Figure S10A**). The peak potentials were found to be unaltered but a slight decrease in peak currents were observed. As compared to the initial current measured on day 1, peak currents on day 3 and day 6 decreased to 94% and 90% respectively (**Figure S10B**). These decrease are near negligible which show that germanane modification on GC electrodes have relatively high stability. Additionally, the effect of pH on the detection properties of DA were investigated. **Figure S11** present voltammograms of germanane modified electrodes in the presence of DA, UA and AA at varying pH of 2, 4, 6, 7, 8 and 10. From the results obtained, pH 6, 7 and 8 have distinct separations of DA peaks from UA and AA, allowing selective detection of DA. These peaks were found at the same potential of +0.64 V. However, peak current in pH 7 is found to be twice as high as that in pH 6 and 8. There are no observable peak separations in pH 2, 4 and 10. This shows that a neutral pH is optimum for detection of DA whereas acidic and alkaline pH diminish the selectivity of DA detection.

Previously, 2D siloxene sheets have been reported as a novel electrochemical sensor for selective dopamine detection. Ramachandran et al.⁵³ showed that these 2D siloxene sheets possessed great improvements in performance as compared to the materials studied herein. This is due to their thin sheet-like nature which directly suggest that size greatly influence the performance of siloxene as material for sensor. Hence, reducing the size and thickness of germanane and methylgermanane could also possibly improve their performance tremendously.

Electrocatalytic Performance

In this section, we report on the electrocatalytic performances of siloxene, germanane and methylgermanane in energy applications, specifically for the oxygen reduction (ORR) and hydrogen evolution reactions (HER). ORR is an essential cathode reaction in fuel cells for energy production while HER is the cathodic half reaction for water splitting in fuel production.^{54,55} These are important reactions in energy applications and several studies aiming at improve their costs and efficiency have been conducted. To date, noble metals such as platinum (Pt) remain to be the best performing catalyst with highest efficiency but are not economically favorable.⁵⁶ As such, the search for cheaper alternatives with similar catalytic efficiency is growing.

Electrocatalytic performance towards ORR was investigated by linear sweep voltammetry (LSV) in KOH (0.1 M). Their performances are compared with the catalytic performance of Pt, more commonly used in the form of Pt/C.⁵⁵ In ambient conditions (solid lines), the cathodic current decreases due to a direct 4-electron reduction pathway from O₂ to H₂O in aqueous solution.⁵⁷ From **Figure 6A**, the respective reduction peaks attributed to ORR were clearly observed. The identities of these ORR peaks were verified by conducting the experiments in N₂-purged conditions (dotted lines) in which the peaks are absent in oxygen-free conditions. The electrocatalytic ORR performances of the materials were analyzed using onset potentials; the potential at which 10% of the maximum current is reached, as presented in **Figure 6B**. Overall, the onset potentials of siloxene, germanane and methylgermanane are higher than Pt/C indicating weaker ORR catalytic performance. Nonetheless, germanane possesses a slightly lower onset potential than bare GC, -0.28 V and -0.29 V respectively but siloxene and

methylgermanane have slightly higher onset potentials than bare GC specifically, -0.32 V and -0.29 V respectively, indicating unfavourable electrocatalytic performance for ORR.

Figure 6. A) Linear sweep voltammograms for ORR in ambient (solid line) and N₂-purged (dotted line) conditions. B) Average onset potentials with respective standard deviations of GC, siloxene, germanane, methylgermanane and Pt/C. All measurements were performed in KOH (0.1M) using Pt counter electrode at a scan rate of 5 mV s⁻¹ vs Ag/AgCl reference electrode.

Next, electrocatalytic performance towards the HER was investigated by LSV in H₂SO₄ (0.5M) (**Figure 7A**) and analyzed by comparing their respective overpotential; that is, the potential at which the current density of -10 mA cm⁻² is achieved. Overpotential is commonly used as a

performance indicator of the HER efficiency in energy applications, with lower overpotentials indicating higher catalytic performance for the HER.⁵⁸ It is clear that Pt/C has the lowest overpotential of -0.14 V indicating superior electrocatalytic performance towards the HER as compared to others. Siloxene and methylgermanane have higher overpotentials than GC indicating poorer electrocatalytic performance for the HER but germanane has a lower overpotential than bare GC, -1.04 V and -1.11 V respectively, suggesting improved electrocatalytic performance for the HER. Similar to the ORR, only germanane showed improvement as the catalyst for the HER in comparison to bare GC. Apart from the HER overpotential, Tafel slopes also evaluate the electrocatalytic ability for the HER and is employed to deduce the rate limiting step of a material. Tafel slopes, b , are obtained by plotting the linear range fitted to the Tafel equation of $\eta = a + b \log |j|$, where η : overpotential and j : current density as shown in **Figure 7B**. Following are possible rate limiting steps of the HER:^{59,60}

- 1) Adsorption (Volmer process): $\text{H}_3\text{O}^+ + \text{e}^- \rightarrow \text{H}_{\text{ads}} + \text{H}_2\text{O}; \quad b \approx 120 \text{ mV dec}^{-1}$
- 2) a. Desorption (Heyrovský process): $\text{H}_{\text{ads}} + \text{H}_3\text{O}^+ + \text{e}^- \rightarrow \text{H}_2 + \text{H}_2\text{O}; \quad b \approx 40 \text{ mV dec}^{-1}$
- b. Desorption (Tafel process): $\text{H}_{\text{ads}} + \text{H}_{\text{ads}} \rightarrow \text{H}_2; \quad b \approx 30 \text{ mV dec}^{-1}$

The first step of the HER is the formation of hydrogen atoms by adsorption followed by either a Heyrovský or Tafel desorption step. Out of the three materials, germanane produces the lowest Tafel slope value of 184 mV dec^{-1} while the Tafel slope of siloxene and methylgermanane are higher, specifically 202 and 303 mV dec^{-1} , respectively. Tafel slopes of all three materials suggest the rate limiting step to be a Volmer adsorption process, similar to that of bare GC (150 mV dec^{-1}). Germanane has the best performance as an electrocatalyst for the HER amongst the three materials.

Figure 7. A) Linear sweep voltammograms for HER and B) Tafel plots of GC, siloxene, germanane, methylgermanane and Pt/C with respective standard deviations. All measurements are performed in H_2SO_4 (0.5M) using bare GC counter electrode at a scan rate of 2 mV s^{-1} vs RHE.

When comparing with other monoelemental materials, siloxene, germanane and methylgermanane have better HER performance than bulk pnictogens but weaker than shear exfoliation pnictogen nanosheets.⁶¹ For ORR, these functionalized group 14 materials possess comparable performance to triple-layered and double-layered graphene but are weaker than few-layered three-dimensional (3D) graphene and graphene with low oxygen content.^{62,63} Other 2D materials commonly reported as HER and ORR catalysts include transition metal dichalcogenides (TMDs) and transition metal oxides. Generally, HER catalytic performance of group 6 (Mo and Ws) TMDs⁶⁴ were reported to be higher whereas HER performance of group 4 (Ti, Zr and Hf)⁶⁵ and group 5 (V, Nb and Ta) TMDs were found comparable with an exception of VTe_2 which exhibited a much lower HER overpotential of 0.5 V.⁶⁶ The ORR catalytic performance of siloxene, germanane and methylgermanane were found comparable with group 6 TMDs and transition metal oxides with an exception from Mn oxides which possessed low onset potentials indicating higher ORR catalytic performance.^{64,67}

Cytotoxicity Studies

Next, toxicity of siloxene, germanane and methylgermanane were evaluated on four cell lines obtained from different parts of a human body, namely breast carcinoma (MCF-7), lung carcinoma (A549), kidney (HEK 293), and liver carcinoma (HepG2) cells. Toxicity is an important property to study prior to large scale usage of new materials^{68,69} and toxicity of these materials have yet to be determined. This serves as a preliminary step towards investigating their biocompatibility for biological applications. Herein, cytotoxicity was assessed by evaluating cell viabilities after exposure to the respective materials in increasing concentrations. Cell viability was measured using cell counting kit-8 (CCK-8); a sensitive colorimetric cell proliferation assay whereby cell viability can be quantifiable by absorbance spectroscopy.⁷⁰

The results obtained show varying toxic properties for different materials in different cell lines (**Figure 8**). Cells exposed to siloxene show no change in cell viability (red bars), which indicate negligible toxicity towards all cell lines. This is expected as several silicon-based materials are similarly reported to be non-toxic.⁷¹⁻⁷³ On the other hand, germanane and methylgermanane are found to induce dose-dependent toxicity on cells. Previously, studies have indeed reported high toxicity of germanium nanoparticles.⁷⁴ Additionally, our studies found germanane to be more toxic than methylgermanane, specifically in low concentrations (6.25 and $12.5 \mu\text{g mL}^{-1}$). This implies that methylation of germanane can reduce toxicity in the lower concentration range. Notably, germanane and methylgermanane were both found to be most toxic towards HepG2 cells and least toxic towards MCF-7 cells. As an illustration, upon exposure of $3.125 \mu\text{g mL}^{-1}$ of germanane, the cell viability reduced to 64% for HepG2 cells while the other cell lines remained above 75%. Likewise, upon exposure of $6.25 \mu\text{g mL}^{-1}$ of methylgermanane to HepG2 cells, the cell viability reduced to 69% while other cells remained above 80%. The

lowest viability of MCF-7 cells remained at 40% despite exposure of the highest concentration (50 $\mu\text{g mL}^{-1}$) of both materials. This implies that germanane and methylgermanane are more toxic towards liver cells and are more compatible towards breast cells. Interestingly, the toxicity trend of the materials in carcinoma cells (A549 and HepG2) and normal cells (HEK 293) are similar.

Figure 8. Percentage of cell viability for A549 cells, HEK 293 cells, HepG2 cells, and MCF-7 cells upon introduction of 0 to 50 $\mu\text{g mL}^{-1}$ of siloxene, germanane and methylgermanane for 24 hours with the respective standard deviations. Cell viability percentages were obtained from normalization of absorbance readings with readings of negative control.

Control studies of the respective materials were performed to eliminate assay interference which may lead to false results.^{75,76} They were carried out with materials in the absence of cells to observe false positive and negative results due to interferences from the materials towards the assay reagents. From the control studies, false positive results for germanane and methylgermanane were found. Due to this, a background removal step was implemented before analysis of cytotoxicity data above to obtain more accurate results. To elaborate, **Figure S12A** shows normalized absorbance measurements taken at 450 nm wavelength at different concentrations of materials. From the results, germanane and methylgermanane increased in absorbance with increasing concentration of materials which suggest possible interference. This was confirmed by obtaining absorbance spectra (350-550 nm) of respective materials in different concentrations in both the absence and presence of cells. For siloxene, absence of peaks was observed for the control set while peaks were observed in the presence of cells as expected (**Figure S12B**). This is not the case for methylgermanane (**Figure S12C**) and germanane (**Figure S12D**) as peaks were observed in the spectra during both the absence and presence of cells. This implies that germanane and methylgermanane possess absorbance at these similar wavelengths which resulted in false positive results. This may be due to strong light absorbance in the whole visible light region by germanane and methylgermanane as reported by Liu et al.²⁸

5.3 Conclusion

Siloxene ($\text{Si}_6\text{H}_3(\text{OH})_3$), germanane (Ge_6H_6) and methylgermanane ($\text{Ge}_6(\text{CH}_3)_6$) were successfully synthesized and functionalized as confirmed by characterization studies through SEM, AFM, HR-TEM, EDS, XPS, XRD, FTIR and Raman spectroscopy. Following that, electrochemical properties studies show that they possessed relatively fast heterogeneous electron transfer (HET) rates, compared to a bare GC electrode, which implies improved performance for electrochemical detection. Germanene-based materials were found to be superior to siloxene as they demonstrate faster HET rates and higher sensitivity for detection of dopamine (DA). Between the two germanene-based materials, germanane portrayed the most sensitive detection of dopamine (DA). Similarly, electrocatalytic studies found germanane to have the best electrocatalytic properties towards the oxygen reduction (ORR) and hydrogen evolution (HER) reactions with improved performance to a bare GC electrode. Toxicity studies show negligible toxic properties for siloxene but dose-dependent toxicity for germanane and methylgermanane. Interestingly, it is found that methylation can successfully reduce toxicity of germanene-based materials in a lower concentration range. Overall, germanane-based materials have more superior properties than siloxene, with germanane (Ge_6H_6) as the best material for electrochemical applications. These studies provide essential fundamental insights into the electrochemical properties and toxicity of these functionalized group 14 layered materials for future electrochemical applications.

5.4 Experimental Section

Synthesis of Materials

Siloxene⁷⁷ ($\text{Si}_6\text{H}_3(\text{OH})_3$): CaSi_2 (0.500 g, 5.20 mmol) was treated with 35% aqueous hydrochloric acid (50 mL) at room temperature and stirred for 24 hours. A yellowish solid was then collected by filtration and rinsed with 35% aqueous hydrochloric acid (2×25 mL), water (5×100 mL) and acetone (2×50 mL).

Germanane²² (Ge_6H_6): CaGe_2 (1.000 g, 5.40 mmol) was added to 35% aqueous hydrochloric acid (100 mL) at -35 °C and stirred for 7 days. A grey solid was then collected by filtration, washed with cold 35% aqueous hydrochloric acid (2×25 mL), water (5×100 mL) and acetone (2×50 mL).

Methylgermanane²³ (Ge_6Me_6): CaGe_2 (0.400 g, 2.16 mmol) and methyl iodide (5 mL, 11.4g, 80.32 mmol) were inserted into a sintered glass funnel (pore size S4) and closed with parafilm. The bottom part of the funnel was filled with water and stirred for 7 days. The solid was collected by filtration, washed with water (5×100 mL) and acetone (3×50 mL).

The products were dried in *vacuo* and stored in the dark under an inert argon atmosphere.

Instruments

SEM and EDS was imaged by JEOL 7600F (Japan). Phoibos 100 spectrometer with a monochromatic Mg X-ray radiation source (SPECS, Germany) was used to obtain XPS data and analysis was carried out with CasaXPS software. InVia Raman microscope (Renishaw, England) in backscattering geometry with CCD detector, DPSS laser (532 nm, 50 mW) with applied power of 10 mW was used for Raman spectroscopy measurements. Fourier transform infrared spectroscopy (FTIR) measurements were performed on a iS50R FTIR spectrometer

(Thermo Scientific, USA). μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) was used to conduct electrochemical measurements and analysis were carried out using NOVA version 1.10 software. Thermo Multiskan GO was used to measure absorbance readings and analyses were carried out by SkanIt software for microplate readers (Thermo Fisher Scientific, Singapore).

Electrochemical Measurements

The materials were dispersed in ultrapure water (2.5 mg mL^{-1}) and subjected to 1 hour of ultrasonication to obtain well-dispersed suspensions. Prior to electrochemical measurements, the suspensions were subjected to an addition of 10 minutes of ultrasonication to maintain well-dispersed samples. For electrode modification, $5 \text{ }\mu\text{L}$ of suspensions were drop-cast onto bare GC electrodes and dried under a lamp. Inherent electrochemistry was studied by cyclic voltammetry (CV) in nitrogen-purged conditions at a scan rate of 100 mV s^{-1} in 50 mM , pH 7.0 PBS. Electrochemical sensing of 1.0 mM ascorbic acid, 0.1 mM uric acid and 0.1 mM dopamine was similarly conducted by CV at a scan rate of 100 mV s^{-1} in 50 mM , pH 7.0 PBS. HET studies we conducted by CV with 10 mM ferro/ferricyanide $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox probe at a scan rate of 100 mV s^{-1} in potassium chloride (0.1 M) as supporting electrolyte. The k_{obs}^0 values were calculated by Nicholson's method⁷⁸ which correlates to the obtained ΔE_p values. Electrocatalytic performance towards hydrogen evolution reaction (HER) was investigated by linear sweep voltammetry (scan rate of 2 mV s^{-1} in $0.5 \text{ M H}_2\text{SO}_4$) with bare GC as counter electrode. Electrocatalytic performance towards oxygen reduction reaction (ORR) was studied by linear sweep voltammetry in both purged and ambient conditions (scan rate of 5 mV s^{-1} with 0.1 M KOH). All measurements were conducted relative to Ag/AgCl reference electrode and Pt counter electrode unless otherwise stated.

Cytotoxicity Assessments

Materials were dispersed in ultrapure water ($500 \mu\text{g mL}^{-1}$) and subjected to 1 hour of ultrasonication. Culturing of A549 (Bio-Rev, Singapore), MCF-7, HepG2 and HEK-293 cells (ATCC, Manassas, VA, USA) were at 37°C with 5% CO_2 in cell culture media (CCM). CCM consists of Dulbecco's Modified Eagle medium (DMEM), 10% fetal bovine serum (FBS) (Life Technologies, Singapore) and 1 % penicillin/streptomycin (Capricorn). Cells were seeded (3000 cells per well) in a 96-well plate and cultured for 24 hours. After 24 hours, CCM was removed and replaced with varying concentrations of material suspensions prepared in fresh CCM (100 μL per well) and further incubated for 24 hours. Negative control was cells which were only exposed to CCM and background were materials in CCM in the absence of cells. Subsequently, 10 μL per well of WST-8 reagent (Dojindo, Japan) was added and incubated for another hour for the quantification of cell viabilities. After 1 h, absorbance was measured at 450 nm and absorbance spectrum from 350 nm to 550 nm was obtained.

5.5 Supporting Information

Figure S1. Electron image and EDS mappings of **A)** siloxene, **B)** germanane and **C)** methylgermanane.

Figure S2. AFM images and size profiles of siloxene, germanane and methylgermanane.

Figure S3. HR-TEM images siloxene, germanane and methylgermanane and their corresponding EDS maps. Scale bars represent 1 μm .

Figure S4. (A - C) XPS survey scans and HR-XPS of (D - F) oxygen and (G - I) carbon from siloxene, germanane and methylgermanane.

Figure S5. FTIR spectra of **A)** siloxene, **C)** germanane and **E)** methylgermanane and Raman scans of **B)** siloxene, **D)** germanane and **F)** methylgermanane.

Figure S6. FTIR spectra of methylgermanane, germanane and siloxene before and after storage in water for one week.

Figure S7. Raman spectra of methylgermanane, germanane and siloxene before and after storage in water for one week.

Table S1. Summary of peak-to-peak separations (ΔE_p) and HET rate constants (k_{obs}^0) of GC, siloxene, germanane and methylgermanane.

	ΔE_p (mV)	k_{obs}^0 (cm s ⁻¹)
GC	365	1.18×10^{-4}
Si ₆ H ₃ (OH) ₃	248	5.72×10^{-4}
Ge ₆ H ₆	155	2.01×10^{-3}
Ge ₆ Me ₆	138	2.57×10^{-3}

Figure S8. (A) DPV profile with germanane modified electrode at different concentrations of DA (30, 50, 70, 100 μM). (B) Plots of the current as a function of DA concentration with corresponding standard deviation.

Figure S9. (A) CVs with germanane modified electrode at different scan rates. (B) Plot of current against square root of scan rate.

Figure S10. (A) DPV profile of germanane-modified electrode with DA (100 μM) stored for different duration of days. (B) Percentage current change of modified electrodes after 1, 3 and 6 days of incubation.

Figure S11. Cyclic voltammograms (CVs) of germanane-modified electrodes with simultaneous addition of AA (1.0 mM), UA (0.1 mM) and DA (0.1 mM) at different pH values. All measurements are performed using Pt counter electrode at scan rate 100 mV s^{-1} vs Ag/AgCl reference electrode in PBS (50 mM).

Figure S12. (A) Percentage absorbance of siloxene, germanane and methylgermanane in cell-free conditions in the presence of CCM and CCK-8 assay measured at wavelength of 450 nm. Absorbance spectra of (B) siloxene, (C) methylgermanane and (D) germanane in the presence and absence of cells respectively from wavelength of 350 to 550 nm.

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PART II : Layered Materials in Biomedicine

Chapter 6

Layered Graphene Oxide for Drug Delivery

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6.1 Introduction

Graphene and graphene oxide have attracted great interest in the scientific community since the first isolation of graphene from graphite in 2006.¹⁻¹¹ Both graphene and graphene oxide possess unique properties such as high mechanical strength, excellent electrical and thermal conductivity, fast mobility of charge carriers and large surface area to volume ratio.⁴ Unlike graphene, graphene oxide has oxygen functional groups on its surface which improves several of its properties such as high solubility, dispersibility and biocompatibility.^{3,8} Due to this, graphene oxide has been utilized in many applications such as in electronics¹², catalysis¹³, sensing^{7,14-16}, optical¹⁷, energy and environmental¹⁸ and biomedicine^{2,3,19}.

Specifically, graphene oxide (GO) has been reported to display great potential for drug delivery and cancer therapy. For instance, Feng *et al.*¹⁹ developed graphene-based self-assembled nanosystem for effective loading, sustained released of doxorubicin (DOX) and enhanced biocompatibility by functionalization with naphthalene-terminated PEG (NP). This nanosystem successfully demonstrated long-term stability, effectively mediated cellular uptake of DOX and potentiated toxicity of DOX against cancerous HeLa cells. Sun *et al.*²⁰, on the other hand, reported on pegylated nano-graphene oxide (NGO-PEG) as drug carriers for DOX and observed promising properties for drug delivery and live cell imaging such as solubility, compatibility, photoluminescent, large specific surface area, low cost and many more. In another study, Zhang *et al.*²¹ reported on functionalized nanoscale graphene oxide (FA-NGO) as a novel nanocarrier for loading and targeted delivery of mixed anticancer drugs, doxorubicin (DOX) and camptothecin (CPT). This study demonstrated specific targeting and high cytotoxicity of FA-NGO/CPT/DOX to human breast cancer (MCF-7) cells. Generally, most studies functionalize GO prior to attachment of drugs for drug delivery to achieve enhancements of properties for drug delivery. However, few studies reported GO as drug

carrier without prior functionalization. An example is a study by Barahuie *et al.*²³ whereby they reported GO as a nanocarrier for controlled release and targeted delivery of the anticancer active agent, chlorogenic acid. Chlorogenic acid-graphene oxide nanocomposite (CAGO) was found to have high thermal stability, possess insignificant toxic effect towards non-cancer cells and portray enhanced toxic effects towards cancer cells as compared to GO and CA in free forms. In general, many studies have reported on the development of GO as drug carriers with enhancements in biocompatibility, cellular uptake of drugs and specific targeting by additional functionalization.¹⁹⁻²³ However, despite vast research on GO, anticancer properties and drug carrier capabilities of non-functionalized graphene oxide have yet to be fully explored.

Figure 1. Chemical structure of cisplatin.

Different preparation methods of GO can exhibit different influence on its properties.^{24,25} Hence, to expand the understanding of other types of GO in drug delivery and cancer therapy, we investigated the toxicity and drug potentiation potential of uniquely prepared graphene oxide nanoplatelets (GONPs) with a platinum drug, cisplatin. Cisplatin (CP), or cis-diamminedichloroplatinum (II) with its chemical structure shown in Figure 1, is a well-known chemotherapeutic drug for various cancer conditions including bladder, head and neck, lung and ovarian cancer.²⁶ Previously, a similar toxicity and drug potentiation study was conducted on graphene quantum dots (GQDs) and they observed an enhancement of anticancer activity of CP by GQDs as a result of increased cellular and nuclear uptake of CP.²⁷

GONP was synthesized, characterized and studied by our group in various applications such as labels for biomolecule recognition for single nucleotide polymorphism detection²⁸, mycotoxin aptasensing amplification²⁹, thrombin aptasensing³⁰ and specific protein-target recognition³¹. Specifically, the well-defined reduction signal of GONP was used for detection of DNA hybridization and polymorphism in a simple and rapid manner using disposable screen-printed electrodes²⁸, detection of ochratoxin A (OTA) in the concentration range of 310 fM to 310 pM with selectivity against ascorbic acid interference²⁹ and detection of thrombin in the concentration range of 3 pM to 0.3 μ M with good selectivity against interferences such as IgG, avidin and BSA³⁰. Hence, with great performance of GONP in detection of biomolecules, we expanded the research of GONP for other biological applications.

GONP was prepared according to our previous works²⁸⁻³¹ from commercially available, well-defined stacked graphite nanofibers (SGNF) with base of 50 x 50 nm. SGNFs were oxidized and consequently laterally unzipped to form the desired graphene oxide nanoplatelets (GONP) before co-administrating them with cisplatin (CP) to treat the cells. This process is schematically represented in Figure 2. This study aims to investigate the potential of GONP to deliver CP into human lung cancer cells (A549) as well as to determine any synergistic potentiation of anticancer effect of CP with GONP.

Figure 2. Schematic representation of SGNF undergoing oxidation and lateral unzipping forming GONP followed by the loading of cisplatin onto GONP forming GONP-CP before treating A549 cells.

6.2 Results and Discussion

Material Characterization

Prior to further assessments, a detailed characterization is important to understand the morphology, size, thickness and chemical composition of materials on hand as these properties can highly influence the way they interact with the surrounding environment. Hence, several characterization studies were conducted on GONP prior to investigating its anticancer and drug delivery properties. GONP was characterized using scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy.

Figure 3. SEM (left) and STEM (right) image of GONP.

The SEM image in Figure 3 presented GONP as stacked sheets, an arrangement which is consistent with a layered structure as supported by STEM shown also in Figure 3. More thorough characterizations have been conducted in previous studies by our group.^{24,28,41} Higher resolution SEM image of GONP (also known as SGNF-OX) showed graphene sheets which were randomly distributed similar to Figure 3.²⁴ Additionally, by atomic force microscopy (AFM), GONP was observed to have an average thickness of 1.2 nm.²⁸ Following that, Raman spectroscopy was performed revealing two characteristic peaks at the wavelengths of about

1350 cm^{-1} and 1580 cm^{-1} (Figure 4). 1350 cm^{-1} , commonly known as D peak, corresponds to a disorder band that is associated to sp^3 -based defects in the graphene sheets whereas 1580 cm^{-1} , commonly known as G peak, corresponds to a peak associated to sp^2 -hybridized carbon.^{24,32} These two peaks are commonly reported characteristic peaks for graphene oxide nanoplatelets (GONP).³³

Figure 4. Raman spectrum of GONP.

Subsequently, energy dispersive X-ray spectroscopy (EDS) and X-Ray photoelectron spectroscopy (XPS) were performed on GONP-CP so as to evaluate successful binding of the drug, cisplatin, $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ (Figure 1), onto GONP. EDS maps of GONP-CP (Figure 5) showed well distributed carbon (C) and oxygen (O) elements on the surface of the GONP. C and O are characteristic elements for graphene oxide.³⁴ In addition, platinum (Pt), chlorine (Cl) and nitrogen (N) are also found distributed on the surface where the nanomaterial can be found suggesting a possible interaction between cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$) with GONP.

Figure 5. STEM image and EDS mappings of GONP-CP.

Following that, XPS was performed on both GONP and GONP-CP to determine their elemental surface compositions and chemical bonding. Figure 6 shows XPS C 1s core-level spectra of GONP and GONP-CP. From the GONP XPS spectrum, peaks at binding energies 284.6, 286.5 and 287.8 eV were observed. The most prominent peak was observed at binding energy of 284.6 eV and it corresponded to sp^2 C=C bond. Binding energies at 286.5 and 287.8 eV correspond to C-O and C=O bonds respectively, indicating the presence of hydroxyl/epoxide and carbonyl groups respectively. These values are characteristic peaks present on the surface of GONP nanomaterials and are comparable to those found in literature.^{24,35} These suggests successful preparation of GONP from SGNF. After introducing CP to GONP (GONP-CP), XPS was also performed to evaluate changes on their elemental surface compositions and chemical bonding. GONP-CP XPS spectrums possessed peaks at binding energy of 284.4, 286.6, 288.5, and 291.5 eV. The most prominent peak still belonged to sp^2 C=C bond at binding energy of 284.4 eV while the binding energies of 286.6 and 288.5 eV which corresponds to C-O and C=O bonds decreased in peak intensity significantly as compared to that in GONP. This suggests that the introduction of CP reduced the amount of oxygen-containing species on

GONP. Also, an additional peak belonging to $\pi - \pi^*$ signal at binding energy of 291.5 eV appeared indicating that the delocalized p conjugation, similar to that of graphene, was restored.³⁶ This $\pi - \pi^*$ signal is assigned to π electrons that are delocalized at the aromatic network in graphene but disappears upon oxidation.³⁷ This shows that the introduction of CP to GONP both reduced the amount of oxygen-containing species and restored the delocalization of π electrons of graphene in GONP. These differences between XPS spectrum of GONP and GONP-CP suggest a possible interaction between CP and GONP.

Figure 6. High resolution XPS C 1s core-level spectra of GONP and GONP-CP.

Cytotoxicity assessments

Toxicity studies on graphene and its derivatives have been widely studied in recent years.^{6,38-42} Various discussions on the toxicity of graphene oxide have been conducted and in general, graphene oxide is found to be non-toxic.⁴³⁻⁴⁶ Herein, the cytotoxicity of GONP and GONP with CP (GONP-CP) was evaluated in human lung cancer cell line (A549) upon exposure for 24 hours. A549 was selected due to clinical relevance of cisplatin in the treatment of human lung cancer.²⁶ Prior to cytotoxicity assessment of GONP-CP, it is necessary to investigate the cytotoxicity of GONP to confirm the non-toxic behavior of graphene oxide as reported in literature. GONP was used in the form of colloidal solution in ultrapure water.

Figure 7. Cell viability of A549 cells measured by WST-8 assay after 24 hours exposure to varying amounts of GONP and GONP-CP. The percentages are normalized to the data obtained from the control set that was exposed to 0 µg/mL nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

The cell viability results after 24 hours exposure to GONP and GONP-CP are presented in Figure 7. A549 was treated with GONP (blue bars) and GONP-CP (red bars) in the concentration range of 0 to 30 $\mu\text{g/mL}$ for 24 hours before conducting cell viability measurements by water-soluble tetrazolium salt (WST-8) assay. WST-8 assay is a well-known assay with higher sensitivity as a chromogenic indicator for cell viability compared with conventional tetrazolium salts.⁴⁷ From the results, we observed GONP itself exhibiting negligible cytotoxicity across all tested concentration as cell viability remained relatively high (above 92%). These results are in agreement to a study published by Chng *et al.* whereby GONP was similarly reported to have relatively low cytotoxicity in the same concentration range (0 to 30 $\mu\text{g/mL}$) when evaluated with WST-8 assay.⁴¹

Subsequently, we co-administered the same concentration of the drug, CP (4.5 $\mu\text{g/mL}$), with similar concentrations of GONP (0 to 30 $\mu\text{g/mL}$) into A549 for 24 hours (as represented by red bars). Initially, with 0 and 2.5 $\mu\text{g/mL}$ of GONP-CP, a drastic drop of cell viability to 63% was observed and this drastic decrease in cell viability could be primarily due to the chemotherapeutic effect of 4.5 $\mu\text{g/mL}$ of cisplatin. Subsequently, an unexpected dose-dependent toxicity on A549 significantly occur upon treatment with 5, 10 and 30 $\mu\text{g/mL}$ of GONP-CP in which the cell viability dropped to 49%, 31% and 13% respectively. This dose-dependent toxic effect of was not observed previously when cytotoxicity of GONP itself was assessed with the same concentrations. In short, we obtained a potentiated toxicity effect of the same concentration of CP when it is co-administered with GONP despite GONP itself displaying negligible toxicity. These results possibly suggest that the presence of GONP while administering CP allowed a higher amount of cellular intake of CP into the cells as compared to when treated with CP only. Hence, we observed an increase in chemotherapeutic efficiency of CP.

Figure 8. Cell viability of A549 cells measured by WST-8 assay after 24 hours exposure to varying amounts of CP and CP-GONP. The percentages are normalized to the data obtained from the control set that was exposed to 0 µg/mL nanomaterials. The results were obtained from a mean of three repeats with their calculated $\pm$ standard deviations.

In the next study, we investigated the toxicity of CP-GONP with varying concentration of CP and compared with toxicity of varying concentration of CP itself. We co-administered varying concentrations of CP (0 to 20 µg/mL) with a constant concentration of GONP (15 µg/mL) labeled as CP-GONP and treated A549 cells for 24 hours before measuring the cell viability by WST-8 assay. Treatment of A549 with varying concentrations of CP itself (0 to 20 µg/mL) was also tested for comparison. These data are presented in Figure 8.

From the results obtained, CP itself (orange bars) presented dose-dependent toxicity on A549. 1 µg/mL of CP does not have any significant toxicity on A549 as the cell viability remains relatively high at 98.5% but as the concentration of CP increased to 5, 10 and 15 µg/mL, cell

viability further decreased to 50%, 40% and 37 % respectively. These results are in agreement with a study by Sui *et al.*²⁷ in which they reported cell viability of A549 after exposure to cisplatin (CDDP). Sui et al. reported that upon treatment with 3.3 μM ($\approx 1 \mu\text{g/mL}$) of CDDP, cell viability of A549, similarly, remained relatively high at 98% and upon treatment with 16.67 μM ($\approx 5 \mu\text{g/mL}$) of CDDP, cell viability reduced to approximately 50%.

CP-GONP, like GONP-CP, was successful in increasing the chemotherapeutic effect of CP. Upon treatment with 1 $\mu\text{g/mL}$ of CP-GONP, cell viability of A549 significantly reduced to 83.8%, as compared to 98.5% when treated by CP itself. Furthermore, when treated with 5, 10 and 15 $\mu\text{g/mL}$ of CP-GONP, cell viability further reduced to 19%, 16% and 15% as compared to 50%, 40% and 37 % when treated by CP itself at the respective concentrations. However, cell viability plateaued out above with 5 $\mu\text{g/mL}$ of CP-GONP as the toxic effect may be limited by concentration of GONP. However, the toxic effect of CP was potentiated at every tested concentration of CP-GONP. These results suggest that co-administrating CP and GONP increases the chemotherapeutic efficiency of CP which means that a smaller amount of CP to achieve higher toxic effect.

Statistical analysis

We conducted statistical analysis on the cell viability results so as to determine its statistical significance. The data was subjected to *t* test (GraphPad) and their p-values were obtained and by convention, $p < 0.05$ shows that the two data sets are statistically significant. When comparing between GONP and GONP-CP, $p \leq 0.001$ for all concentrations and when comparing between CP and CP-GONP, $p < 0.05$ from concentration of 2.5 $\mu\text{g/mL}$ to 20 $\mu\text{g/mL}$. These suggest that the data presented in Figure 7 and Figure 8 are statistically significant in the mentioned concentration range.

6.3 Conclusion

In conclusion, we have successfully synthesized and characterized graphene oxide nanoplatelets (GONP) from stacked graphite nanofibers (SGNF). Cytotoxicity assessments revealed negligible toxicity of GONP between concentrations of 0 and 30 $\mu\text{g/mL}$. Upon co-administration of constant concentration of CP with varying concentration of GONP (GONP-CP), an increase in toxicity was observed when treated with above 5 $\mu\text{g/mL}$ of GONP despite negligible toxicity of GONP. Similarly, upon co-administration of varying concentration of CP with a constant concentration of GONP, an increase in toxicity was observed upon treatment with every tested concentration of CP. These results suggest that co-administrating CP with the as-synthesized GONP results in a synergistic toxic effect on A549. Possibly, this could be due to higher amount of cellular intake of CP into A549 in the presence of GONP. Also, these means that treatment with smaller amount of drug, CP, is needed to achieve higher toxic effect on A549 as compared to when treated with CP only. In short, not only was GONP found non-toxic to A549, but we have shown that co-administrating CP with GONP could potentiate the anticancer effect of CP in the treatment of human lung cancer cells (A549). Thus, the as-synthesized GONP could be a remarkable material for various drug delivery and cancer therapeutic applications in future.

6.4 Experimental Section

Nanomaterials and Chemicals

Stacked graphite nanofibers were purchased from STREM Chemicals (Newburyport, MA, USA). Sulfuric acid (H_2SO_4), potassium permanganate (KMnO_4) and sodium nitrate (NaNO_3) were purchased from Sigma-Aldrich, Singapore. All solutions were made up using Milli-Q water with $18.2 \text{ M } \Omega \text{ cm}$ resistivity (ultrapure water).

Cell Viability Assays and Culture Media

The cell viability assay, water-soluble tetrazolium salt assay (WST-8 assay), was obtained from Dojindo. The cell culture media (CCM) were comprised of Dulbecco's Modified Eagle medium (DMEM) from Gibco, fetal bovine serum (FBS) from PAA Laboratories and 1% penicillin-streptomycin liquid from Capricorn. Phosphate buffer solution (PBS) of pH 7.2 was purchased from Gibco.

Instruments

Scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM) and energy dispersive X-ray spectroscopy (EDS) were carried out using a Jeol 7600F instrument (Jeol, Japan). X-ray photoelectron spectroscopy (XPS) measurements were performed using Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). Absorbance readings were measured by Thermo Multiskan GO (Thermo Fisher Scientific).

Synthesis methods of Graphene Oxide Nanoplatelets

Graphene oxide nanoplatelets (GONP) were previously prepared and characterized in details according to our previous studies.²⁸ In short, stacked graphite nanofibers (SGNF) was used as starting materials to prepare GONP by first forming graphite oxide nanofibers (GONF) through oxidation of SGNF with a mixture of NaNO_3 , H_2SO_4 and KMnO_4 . Following that, the obtained GONF was dispersed in ultrapure water and ultrasonicated for 3 hours to acquire GONPs.

Cell Culture

Human lung epithelial cell line (A549) was obtained from Bio-REV Singapore and have a typical cell cycle of 22 hours. A549 was cultured in a cell culture medium which is made up of a mixture of DMEM, 10% FBS and 1% penicillin-streptomycin liquid, in an incubator of 37°C under 5% CO_2 . 5000 cells were seeded per well (100 μL) in a 96-well plate for 48 hours.

Nanomaterials introduction to cells

GONP was prepared in ultrapure water at a concentration of 300 $\mu\text{g/mL}$ and subjected to ultrasonication for 30 minutes to attain a well-dispersed suspension. Prior to each test, GONP suspensions undergo ultrasonication for another 10 minutes to maintain the homogeneous dispersion of the sample. GONP-CP was prepared in by mixing various concentrations of GONP (0 – 30 $\mu\text{g/mL}$) and constant concentration of CP (4.5 $\mu\text{g/mL}$) in ultrapure water for 24 hours. After 24 hours, each mixture was topped up to 100 μL per well with CCM before treating the cells. CP-GONP was prepared by mixing various concentration of CP (0 to 20 $\mu\text{g/mL}$) and constant concentration of GONP (15 $\mu\text{g/mL}$) in ultrapure water for 24 hours. After 24 hours, each mixture was topped up to 100 μL per well with CCM before treating the cells.

After 48 hours incubation of the seeded cells, the CCM per well was removed and treated with various concentration of GONP, GONP-CP and CP-GONP suspensions. Cells with no nanomaterials were used as a negative control. The cells and nanomaterials dispersion (100 μ L per well) were incubated at 37°C under 5% CO₂. This protocol was adapted and modified from our previous report.⁴⁸

WST-8 cell viability assay

After incubating the cells with the nanomaterials for 24 hours, 10 μ L of stock WST-8 reagent was introduced and further incubated (37°C and 5% CO₂) for 4 hours. After 4 hours, absorbance measurement at 450 nm and spectrum absorbance measurement (400 nm to 500 nm) of every concentration were obtained.

WST-8 control experiment

Nanomaterials of varying concentrations (0 to 200 μ g/mL) were incubated (37°C and 5% CO₂) in cell culture media for 24 hours. After 24 hours, similar to cell viability assessment, 10 μ L of stock WST-8 reagent was introduced and further incubated for 4 hours. After 4 hour, absorbance measurement at 450 nm and spectrum absorbance measurement (400 nm to 500 nm) of every concentration were obtained. The absorbance values obtained from the control were deducted from the absorbance values of cell viability measurements to remove background and interferences.

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Chapter 7

Layered Arsenene as Drug Carriers

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7.1 Introduction

Nanotechnology has allowed rapid advancements in several fields of research. Nanoscale materials possess several favourable characteristics such as superior electronic properties, high specific surface area, high loading capabilities, high cellular uptake and various surface functionalities, high mechanical flexibility and etc.^{1,2} One area of nanotechnology that have benefitted from these properties is nanomachines, which are nano-sized structures capable to convert other forms of energy into motion.³ Commonly, nanomachines rely on on-board catalysts to convert local chemical fuel in a solution into motion.⁴ These nanomachines are known to be useful for many biomedical applications including; sensing, drug delivery, nanosurgery and imaging.⁵ In drug delivery specifically, nanomachines have been widely reported to load, carry and transport chemotherapeutic drugs.^{6,7} A diverse types of materials as nanomachines have been reported such as TiO_2 , CaCO_3 , metal-organic frameworks (MOFs), graphene and its allotropes as well as transition metal dichalcogenides (TMDs).⁸

The research into elemental nanomaterials beyond graphene have been trending in recent years.^{2,9} Increasing research on black phosphorus, for instance, have inspired studies on other group V element materials, also known as pnictogens. Pnictogens have semiconducting electronic properties and have shown potential in various applications such as electrochemical catalysis, batteries, biosensing as well as biomedical and bioimaging applications.¹⁰⁻¹⁴ Utilizing pnictogens in biomedical applications is not new. For instance, arsenic sulfide (As_4S_4), also known as realgar, have been reported for the various cancer treatments both *in vivo* and *in vitro*.¹⁵ Antimonene and black phosphorus quantum dots were reported as effective agents for photothermal cancer therapy.^{16,17} Also, bismuth-based microrobots were reported to load, transfer and release anticancer drug doxorubicin (DOX).¹⁸

However, pnictogen nanomachines for biomedicine is currently still in infancy. Hence, we set forth to study two-dimensional (2D) layered arsenene as nanomotors for drug delivery applications. Arsenic are heavy pnictogens which are most stable in its rhombohedral layered structure (β -form), also known as gray arsenic.¹⁹ This rhombohedral structure consists of puckered six-membered ring atoms, stacked similarly to graphene sheets in graphite but held by much stronger bonds due interactions of atomic orbitals between individual double layers. These interlayer interactions cause arsenic to have a semimetal behaviour with a metallic sheen and good electron conductivity.¹⁹ On the other hand, arsenene possess buckled honeycomb structure as the most stable allotropic form.²⁰ Arsenene are obtained by exfoliation of the bulk, gray arsenic. Arsenene have indirect band gap which can be easily tuned by applying strain and is favourable for electronics and optoelectronics.^{21,22} To date, studies on applications of arsenene are only limited to gas sensing and biosensing.^{13, 23-26}

Herein, arsenene nanoparticles (As) are modified with platinum (Pt) to form Janus As-Pt nanomotors. Firstly, their motion capabilities in the presence of H_2O_2 as fuel were studied. Following that, biocompatibility and drug loading competency of these As-Pt nanomotors were investigated. The results showed that As-Pt nanomotors are fast, biocompatible, possess high drug loading and high cellular uptake into cells. These findings represent preliminary research on arsenene nanoparticles as non-toxic self-propelled drug carriers, see **Scheme 1**.

Scheme 1. Self-propelled Janus As-Pt/DOX nanomotors and their application as anticancer drug carrier.

7.2 Results and Discussion

Figure 1. (A and B) SEM micrographs of the As-Pt nanomotor with varying magnifications. (C) EDS elemental maps and (D) spectrum from STEM micrographs of As-Pt nanomotors. (E) DLS from the water solution of the nanomotors.

Material Characterization

Material characterization were first conducted to confirm the success synthesis as well as to provide details on the morphology, size and elemental composition of As-Pt nanomotors. Scanning electron microscopy (SEM) images (**Figure 1A and 1B**) presented flake-like structures with uneven edges. In addition, the surface of the material appears to possess an uneven morphology consistent with layered arrangements. Elemental maps provided by energy dispersive spectroscopy (EDS) (**Figure 1C**) showed homogeneous distribution of arsenic (As) and platinum (Pt) confirming successful evaporation of Pt on As nanosheets. This is supported by EDS spectrum (**Figure 1D**) which showed the presence of As and Pt peaks. Carbon (C) peak was present due to the substrate used in the measurements. The size distribution of As-Pt nanomotors as obtained by dynamic light scattering (DLS) ranges from 160 nm to 250 nm with the average size of 202 nm (**Figure 1E**). X-Ray photoelectron spectroscopy (XPS) was also employed to confirm elemental oxidation states and composition of As-Pt nanomotors. From the survey scan, the presence of As and Pt are confirmed (**Figure 2A**). In addition, oxygen (O) and C were also observed due to possible oxidation of material and contamination with adventitious carbon respectively. Silicon (Si) was detected due to the usage of silicon wafer as XPS substrate. High resolution XPS (HR-XPS) was measured for As, Pt and O elements for a more in depth analysis of respective elemental compositions. As and Pt elements are further confirmed on the nanomotors from the obtained As 3d and Pt 4f HR-XPS scans. From **Figure 2B**, binding energy of As 3d_{5/2} and As 3d_{3/2} spin orbitals were observed at 42.6 eV and 43.3 eV, respectively, whereas from **Figure 2C**, peaks at 70.5 eV and 73.8 eV correspond to Pt 4f_{7/2} and Pt 4f_{5/2} respectively. However, oxidation of the material can be clearly observed. **Figure 2B** present peaks centered at 45.1 eV and 45.8 eV which corresponds to As 3d_{5/2} and As 3d_{3/2} of As₃O₅ while **Figure 2C** show peaks at 73.1 eV and 76.4 eV which corresponds to Pt 4f_{7/2} and Pt 4f_{5/2} of PtO. HR-XPS of O 1s was also obtained to confirm the respective oxidation

peaks (**Figure 2D**). **Figure 2D** show two distinct peaks at 530.7 eV and 531.8 eV which correspond to O 1s of PtO and As₃O₅ respectively. This is expected as it was arsenene was previously reported to easily undergo oxidation as O₂ serves as a strong acceptor to arsenene.^{27,28}

Figure 2. (A) XPS survey scan of As-Pt nanorobots and high resolution-XPS (HR-XPS) (B) of As 3d, (C) Pt 4f and (D) O 1s.

Propulsion of Janus As-Pt nanomotors

Following that, we set forth to study the motion of Janus As-Pt nanomotors. In the presence of H_2O_2 , As-Pt nanomotors were seen to propel in a circular motion (**Figure 3A-3H and Videos S1-S2**). The distance of the motion within a stipulated time was found to vary as the concentration of H_2O_2 changes, implying varying velocities achieved. These velocities with respect to H_2O_2 concentration are summarized in **Figure 3I**. At a significantly low concentration of 0.25 % H_2O_2 , Janus As-Pt nanomotors propel at a velocity of $3 \mu\text{m s}^{-1}$. As expected, the velocities gradually increase as the concentration of H_2O_2 increase. A sharp increase in velocity, from $4.8 \mu\text{m s}^{-1}$ to $8.1 \mu\text{m s}^{-1}$, was observed from as H_2O_2 changes from 1% to 3%. As expected, the highest velocity was seen with the highest H_2O_2 concentration at velocity of $13.7 \mu\text{m s}^{-1}$. Due to the lack of bubble produced, the mechanism of As-Pt nanomotors is highly likely to be self-diffusiophoresis, which is commonly associated with asymmetric catalytic nanomotors. It occurs when decomposed products is generates a concentration gradient across the nanomotor.^{4,5} In this case, the Pt side of the nanomotors can catalyze a reaction with H_2O_2 , causing a concentration gradient of products across the Pt side and As side, leading to a propulsion.

Figure 3. (A - H) Tracking images of As-Pt nanomotors trajectory at different H_2O_2 concentrations. (I) Dependence of As-Pt nanomotors velocity with varying H_2O_2 concentrations. Scale bar represents 10 μm .

Janus As-Pt nanomotors interaction with A549 cells

In recent studies, nanomotors have been explored in several drug delivery techniques. In this study, Janus As-Pt nanomotors were explored as anticancer drug carriers. Prior to application studies, toxicity and interaction of Janus As-Pt nanomotors in human cells were investigated. The prepared nanomotors were exposed to human lung carcinoma cells (A549) for 24 hrs and their interactions with the cells were studied. The as-prepared Janus As-Pt nanomotors were obtained and diluted to obtain lower concentrations of the materials. 0 dilution corresponds to the stock solution with highest concentration while 32 dilution corresponds to the materials with the lowest concentration after diluting the stock solution 32 times. After incubation of materials in A549 cells for 24 hours, microscopy images revealed several As-Pt nanomotors to have entered the cells (**Figure 4A and 4B**). These observations are very distinct at 0 dilution (**Figure 4A**) but still observable after 32 dilutions of the nanomotors (**Figure 4B**). Control microscopy images of negative control (NC) and positive control (PC), which corresponds to a control with toxic organic compound (10% dimethyl sulfoxide (DMSO)), as well as images of As-Pt nanomotors at the respective dilutions are presented in **Figure S1**. This shows that Janus As-Pt nanomotors possess high cellular uptake by A549 cells which opens up vast possibilities for its applications in biomedicine. Following that, cell viability studies were conducted to study cytotoxic properties of the materials. The nanomotors were prepared in range of dilutions (0, 2, 4, 8, 16 and 32 times) and subsequently introduced to A549 cells for 24 hours prior to measuring their respective cell viabilities. The results are presented in **Figure 4C**. Negative control (NC) corresponds to a control with no nanomotors while positive control (PC) corresponds to a control with 10% DMSO to ascertain low cell viability in the presence of toxic substance. From the results obtained, the lowest cell viability of 24% was observed with introduction of 0 dilution of materials. Interestingly, diluting the materials twice reduced toxicity significantly as cell viability remained high at 66% whereas diluting the materials

further successfully kept cell viabilities above 85% which imply negligible cytotoxicity. This shows that with dilution, As-Pt nanomotors possess low toxicity to human cells which implies possible biocompatibility which is preferred for drug carriers. These results are in contrast to a previous study whereby it was reported that dose-dependent toxicity was exhibited by shear exfoliated arsenene and is the most toxic amongst the tested pnictogens; arsenene, antimonene and bismuthene.²⁹ However, Chou et al. reported varying toxicity level for different forms of arsenicals depending on their chemical form and oxidation states.³⁰ For instance, toxicity of As^{3+} is almost 25 times higher than As^{5+} in terms of LD_{50} values. Therefore, it is likely that As-Pt nanomotors possess low toxicity levels due to difference in its chemical form and structure. In addition, the amount of H_2O_2 used to mobilize As nanomotors is very low such that it does not pose any toxic effects on cells. In previous studies, concentration of H_2O_2 below $10 \times 10^{-6} \text{M}$ was reported to satisfy the biological safety requirements.^{31,32} Hence, the amount of H_2O_2 used will not negatively affect the human body.

Figure 4. Microscopic images of A549 cells after treatment with (A) 0 and (B) 32 times dilution of As-Pt nanomotors for 24 hours. (C) cell viability percentage after cell incubation with varying dilution factors of As-Pt nanomotors. NC is negative control with no nanomotors and PC is the positive control with 10% DMSO for 24 hrs. Scale bar represents 50 μm .

Janus As-Pt nanomotors as drug carriers

Since As-Pt nanomotors presents significant promise in biomedicine due to its high cellular uptake and low cytotoxicity, we set forth to explore its properties as drug carriers. Firstly, drug loading capabilities of As-Pt nanomotors were investigated. As-Pt nanomotors were loaded with doxorubicin (DOX), a potent anticancer agent. As-Pt nanomotors were first exposed to DOX (50 μ M) for 24 hours and subsequently thoroughly washed to remove free DOX from the surface of As-Pt nanomotors. The collected materials are presumed to be As-Pt nanomotors loaded with DOX (As-Pt/DOX). The fluorescence property of DOX (excitation wavelength: 560-570 nm) allows easy imaging and detection of the drug. Hence, the success of binding was confirmed by fluorescence imaging. In **Figure 5A**, white light and red fluorescent microscopic image of As-Pt/DOX can be clearly observed, confirming the successful binding of DOX on As-Pt nanomotors. Additionally, similar microscopic image of A549 cell with successful cellular uptake of As-Pt/DOX was captured. In **Figure 5B**, red fluorescence was observed in the region where As-Pt/DOX was found indicating successful uptake of As-Pt/DOX in A549 cells. These results suggest that the loaded DOX were successfully delivered into cells with Janus As-Pt nanomotors as its carriers, indicating high chemotherapeutic efficacy. Following that, cytotoxicity measurements of Janus As-Pt/DOX was conducted to study its potency on A549 cells. Negative control (NC) with no materials and positive control (PC) with 10% DMSO were also measured. From **Figure 5C**, cell viability of A549 cells drastically reduced to 47% upon introduction of As-Pt/DOX as compared to bare As-Pt which remained high at 86% cell viability. The concentration of As-Pt nanomotors used in both tests were at 4 times dilution from the stock solution as it is the highest concentration of which there is negligible toxicity. However, toxicity of free DOX remains higher with only 23% of cells viable. This shows that toxicity of Janus As-Pt/DOX is almost half as compared to free DOX, possibly due to lesser DOX concentration. This can be overcome by further optimizing the loading of DOX

on Janus As-Pt nanomotors. These results confirm As-Pt nanomotors as promising drug carriers of DOX into human cells.

Figure 5. White-light and fluorescent microscope images of (A) As-Pt nanomotors loaded with DOX and (B) As-Pt/DOX nanomotors into cell. (C) Cell viability percentage incubation with bare As-Pt nanomotors, As-Pt/DOX nanomotors, free DOX (50 μ M), negative and positive control for 24 hrs. Scale bar represents 5 μ m.

7.3 Conclusion

As-Pt nanomotors were prepared successfully and characterized by SEM, STEM, EDS, DLS and XPS. SEM and STEM revealed flake-like, layered morphology and DLS obtained an average diameter of 202 nm of the particles whereas the elemental compositions of As and Pt were successfully confirmed by EDS and XPS. The results from propulsion studies demonstrated fast circular propulsion of As-Pt nanomotors, with H_2O_2 as fuel, by self-diffusiophoresis. Interestingly, at a significantly low concentration of H_2O_2 (0.25%), these As-Pt nanomotors could still successfully move with a speed of $3 \mu\text{m s}^{-1}$. Additionally, As-Pt nanomotors were found to be non-toxic at low concentration, possess high cellular uptake into A549 cells and have promising drug loading capabilities of doxorubicin (DOX). These DOX-loaded As-Pt nanomotors were observed to be uptaken by A549 cells successfully with increased toxicity confirming the success of As-Pt nanomotors as promising biocompatible drug carriers of DOX into human cells.

7.4 Experimental Section

Equipment

JEOL 7600F (Jeol, Japan) were utilised for SEM, STEM and EDS. Thermo Multiskan GO (Thermo Fisher Scientific) and SkanIt software (Thermo Fisher Scientific, Singapore) were used to obtain absorbance readings and analysis, respectively. XPS were performed on Phoibos 100 spectrometer with a monochromatic Mg X-Ray radiation source (SPECS, Germany). CasaXPS software was used to analyse XPS data. Nikon Eclipse TE 2000-E optical microscope connected to Nikon NIS-Elements software was setup to obtained the respective velocities. Optical microscope coupled with filter of excitation wavelength: 560 – 570 nm were utilised to capture drug-loaded Janus As-Pt fluorescence images. Centrifugation was conducted with Beckman Coulter Allegra 64R centrifuge. Zetasizer Nano ZS (Malvern, England).

Preparation of Arsenene-Platinum (As-Pt) Nanomotors

Arsenene nanosheets were prepared from ultrasound exfoliation of arsenic (grey) with purity over 99.9% (Alfa Aesar) for 3 hours. Clean glass slides were prepared by washing with acetone, isopropyl alcohol (IPA) and ultrapure water, in that order, and left to dry in oven. Following that, arsenene nanosheet suspension (300 μL) were dropcasted onto clean glass slides and left to dry for 24 hours. Arsenene (As) nanosheet suspensions (5 mg mL^{-1}) were obtained by dispersing of arsenic (grey) with purity over 99.9% (Alfa Aesar) in ultrapure water by sonication for 1 hour. Thereafter, As modified glass slides were evaporated with platinum (Pt) to form As-Pt nanorobots. Finally, the glass slides were subjected to ultrasonication for 2 minutes in ultrapure water to release As-Pt nanomotors from the glass slides.

Motion Studies of As-Pt Nanomotors

Motion studies of As-Pt nanomotors were conducted in water and hydrogen peroxide (H_2O_2) as fuel. An equal ratio of As-Pt nanomotors solution and H_2O_2 were dropcasted on a glass slide of the optical microscope. Their movements and velocities can be clearly observed and tracked with the Nikon NIS-Elements software. Their average velocities were obtained from at least 10 different velocities obtained.

In-vitro Cytotoxicity Assessments

A549 (Bio-Rev, Singapore) was used for the *in vitro* cytotoxicity assessments of As-Pt nanorobots. Culturing of A549 cells was carried out in cell culture medium (CCM) comprising of 10% fetal bovine serum (FBS), Dulbecco's Modified Eagle medium (DMEM) from Life Technologies, Singapore together with 1 % penicillin/streptomycin from Capricorn. Subsequently, it was incubated with 5% CO_2 at 37°C . 3000 cells per well were seeded in 96-well plates and cultured further for 48 hours. Thereafter, different concentrations of As-Pt nanomotors suspensions were replaced and incubated for another 24 hours. Negative controls were introduced with fresh CCM only and positive controls were exposed with 10% DMSO. Lastly, of WST-8 reagent obtained from Dojindo, Japan ($10\ \mu\text{L}$) was added per well. After 1 hour incubation, absorbance measurements at excitation wavelength of 450 nm were obtained for the quantification of cell viabilities.

Loading of Doxorubicin (DOX) on As-Pt Nanomotors

As-Pt stock solution, which was first obtained from glass slides, were diluted 4 times prior to loading of DOX. Thereafter, $50\ \mu\text{M}$ of DOX were mixed with the diluted As-Pt solution and

left for 24 hours. After 24 hr, centrifugation of mixture (10 000 rpm, 15 minutes) was conducted to collect the DOX-loaded As-Pt particles. Subsequently, the collected particles were washed twice with ultrapure water by vortex, collected again through centrifugation and suspended in ultrapure water before introduction to cells and respective fluorescence imaging measurements.

7.5 Supporting Information

Supporting Videos:

Video S1. Self-diffusiophoresis of Janus As-Pt nanomotors at 5 % H_2O_2 .

Video S2. Self-diffusiophoresis Janus As-Pt nanomotors at 10 % H_2O_2 .

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Figure S1. Microscopy images of cells negative (NC) and positive (DMSO) controls (PC), cells treated with As-Pt nanorobots as well as As-Pt nanorobots only (0 and 32 dilutions factor). Scale bar represents 50 μm .

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Chapter 8

Conclusion and Future Outlook

In summary, we have studied the performance of recently studied layered materials in two different types of applications; electrochemical and biomedicine.

In **Part I**, our investigations on fundamental electrochemistry and electrochemical applications have shown the following findings. Firstly, electrocatalytic studies of platinum dichalcogenides for the oxygen reduction reaction (ORR) have shown platinum ditelluride (PtTe_2) with the most promising performance, comparable with Pt on carbon (Pt/C) that is the ORR's ideal catalyst. Our findings found PtTe_2 more favourable as it is a safer material to handle with possibly lower toxicity towards human respiratory system. Following that, MAX and MAB phases as well as functionalized group 14 layered materials (siloxene, germanane and methylgermanane) were explored for electrochemical applications including sensing of important biomarkers such as ascorbic acid, uric acid and dopamine. In addition, their electrocatalytic performance for important energy reactions in fuel cells including the hydrogen evolution reaction (HER), oxygen reduction reaction (ORR) and oxygen evolution reaction (OER) were also investigated.

Results obtained from safety studies showed a varying toxicity profile of the materials. Generally, MAX and MAB phases were found to be nontoxic on four types of cells; lung, kidney, liver, and breast cancer cells. This implies that they are generally safe to handle and may be potentially biocompatible for future biological applications. However, for group 14 layered materials, negligible toxicity was observed for siloxene but not for germanane and methylgermanane. The two functionalized germanene materials have dose-dependent toxicity but methylgermanane portrayed lower toxicity suggesting that methylation can successfully reduce toxicity of a material. In addition, functionalized TMD, as demonstrated in Chapter 2

(functionalized MoS₂) also showed reduced toxicity upon covalent functionalization with an organic moiety.

These results from **Part I** demonstrated that different elemental compositions, such as different chalcogen atoms on TMDs, different metal element on layered carbides and borides as well as different types of functionalization, are important factors that can affect the electrochemical performance and toxicity profile of the materials.

Part II of the thesis investigated the potential of layered graphene oxide, specifically graphene oxide nanoplatelets (GONP), and layered arsenene, specifically arsenene nanorobots (As-Pt), as drug carriers for anticancer drug delivery applications. These two projects achieved varying aims of layered materials in a drug delivery application. Firstly, our findings show that co-administration of non-toxic GONP with anticancer drug, cisplatin, could potentiate the anticancer effects on cells possibly due to increased cellular uptake of cisplatin into cells in the presence of GONP. On the other hand, As-Pt nanorobots demonstrated fast moving and biocompatible drug carrying potential with high drug loading capacity and cellular uptake. These show the versatility of layered materials for different outcomes in drug delivery applications.

Nevertheless, we recognize that these studies are still in the preliminary stages and more in depth studies are required to fully comprehend all aspects of these layered materials for various electrochemical and biomedical applications. The electrochemical studies can be accompanied with more computational and theoretical studies to fully understand the mechanisms and reactions that are occurring. In addition, synthesis of materials could be better controlled so as

to reduce factors such as oxidation and irregularities in elemental compositions for the experimental studies undertaken in this thesis. For toxicity and drug delivery investigations, the results could be supplemented with *in vivo* studies to provide a more accurate representation of the effects on the human body. In addition, insights on mechanisms for toxicity and drug loading and release would further enhance our understanding on the toxicity profiles and drug delivery process of layered materials in cells.

It is hoped that the fundamental findings obtained from this thesis will still greatly benefit the scientific community by providing new data and insights on several aspects of layered materials.