SURFACE MODIFICATION OF 2-D MXENE AND EVALUATION OF CYTOTOXICITY AND PHOTOTHERMAL THERAPY

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MASTER OF SCIENCE
(BIOLOGY)

UNIVERSITI PERTAHANAN NASIONAL MALAYSIA

2021

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Thesis submitted to the Centre for Graduate Studies, Universiti Pertahanan Nasional Malaysia, in fulfilment of the requirements for the Degree of Master of Science(Biology)

2021

ABSTRACT

The MXenes are a novel family of 2D materials with promising biomedical activity whereas their anticancer potential is largely unexplored. Polyethylene glycol (PEG) surface modified MXenes showed enhanced anticancer potential as compared to Bare MXenes, but, depending on the manufacturing process, PEGs may include ethylene oxide, a known human carcinogen and 1,4-dioxane, a possible human carcinogen. This study aims at investigating poly propylene glycol as a safer alternative to surface modify MXene as it photodegrades less compared to PEG and PPG has been labelled as a safe food additive by the FDA.

In this study, a comparative cytotoxicity investigation for Ti_3C_2MXene, PPG, and PEG surface-modified Ti_3C_2MXene has been conducted towards normal and cancerous human cell lines. The chemical etching method was used to synthesize MXene followed by a simple chemical mixing method for surface modification of Ti_3C_2MXene with PPG and PEG molecules. SEM and XRD analyses were used to examine surface morphology and composition respectively. FTIR and Uv-vis spectroscopy was used to confirm surface modification and light absorption respectively. The cell lines used were normal (HaCaT and MCF-10A) and cancerous (MCF-7 and A375) human cells.

The surface-modified MXenes exhibited a sharp reduction in cell viability towards both normal (HaCaT and MCF-10A) and cancerous (MCF-7 and A375) cells but it was more pronounced towards cancerous cell lines. The highest toxicity towards both normal and cancerous cell lines was observed with PEGylated MXenes followed by PPGylated and Bare MXenes. The normal cell's viability is barely above 70% threshold with 250mg/L PEGylated MXene concentration whereas PPGylated and

Bare MXene are less toxic towards normal cells even at 500mg/L concentration. Moreover, the toxicity was directly related to the type of cell lines. In general, the HaCaT cell line exhibited the lowest toxicity while toxicity was highest in the case of the A375 cell line. The photo-thermal studies revealed high photo-response for PEGylated MXenes followed by PPGylated and Bare MXenes. However, the PPGylated MXenes lower cytotoxicity towards normal cells while comparable toxicity towards malignant cells as compared to PEGylated MXenes makes the former a relatively safe and effective anticancer agent.

ABSTRAK

Mxenes ialah sejenis keluarga baru daripada bahan 2D dengan aktiviti bioperubatan dan sebahagian besar potensi antikansernya belum ditemui.MXenes yang dimodifikasi permukaan dengan polietilena glikol (PEG) menunjukkan potensi antikanser yang meningkat berbanding dengan MXenes yang tidak dimodifikasikan, tetapi, bergantung pada proses pembuatannya, PEG mungkin terkandung etilena oksida, bahan karsinogen manusia yang diketahui dan 1,4-dioksana, bahan yang mungkin karsinogen kepada manusia. Kajian ini bertujuan untuk menyiasat poliil propilena glikol sebagai alternatif yang lebih selamat daripada mengubahsuai permukaan MXene kerana fotodegradasinya kurang dibandingkan dengan PEG dan PPG telah dilabel sebagai bahan tambahan makanan yang selamat oleh FDA.

Dalam kajian ini, penyelidikan sitotoksisiti perbandingan untuk Ti_3C_2MXene PPG, dan PEG permukaan telah diubah ke arah garis sel manusia normal dan barah. Kaedah pengukiran kimia digunakan untuk mensintesis MXene diikuti dengan kaedah pencampuran kimia sederhana untuk modifikasi permukaan Ti_3C_2 MXene dengan molekul PPG dan PEG. Analisis SEM dan XRD digunakan untuk memeriksa morfologi dan komposisi permukaan masing-masing. Spektroskopi FTIR dan UV-vis digunakan untuk mengesahkan modifikasi permukaan dan penyerapan cahaya masing-masing. Garis sel yang digunakan adalah sel normal (HaCaT dan MCF-10A) dan barah (MCF-7 dan A375). MXenes yang diubah permukaan menunjukkan penurunan yang drastik dalam daya maju sel ke arah normal (HaCaT dan MCF-10A) dan sel barah (MCF -7 dan A375) tetapi lebih jelas terhadap garis sel barah. Ketoksikan tertinggi terhadap garis sel normal dan barah telah diperhatikan dengan MXenes PEGylated diikuti oleh MXenes PPGylated dan Bare. Kelangsungan sel normal hampir melebihi

ambang 70% dengan kepekatan MXene PEGylated 250mg / L sedangkan PPGylated dan MXene Bare kurang toksik terhadap sel normal walaupun pada kepekatan 500mg / L. Lebih-lebih lagi, ketoksikan didapati berkaitan secara langsung dengan jenis garis sel.

Secara amnya, garis sel HaCaT menunjukkan ketoksikan terendah manakala ketoksikan paling tinggi dalam kes garis sel A375. Kajian foto-termal mendedahkan tindak balas foto yang tinggi untuk MXene PEGylated diikuti oleh MXenes PPGylated dan Bare. Walau bagaimanapun, PPGylated MXenes menurunkan sitotoksisitas terhadap sel-sel normal sementara ketoksikan yang setanding terhadap sel-sel malignan berbanding dengan PEGylated MXenes menjadikan bahan tersebut sebagai agen antikanser yang agak selamat dan berkesan.

ACKNOWLEDGEMENTS

First, I would like to express my utmost gratitude to the almighty Allah, who has created and erudite me and helped me all the way to finish this work smoothly. Secondly, I am thankful to my parents for their unconditional love and uninterrupted prayers for that what I am today.

I am extremely grateful for the opportunity to work under the supervision of ***Assoc. Prof Dr. Nanthini Siridewi.*** Under her tutelage, I have grown as a student and a researcher as well. She always blessed me with motivation and moral support without which it was very difficult to complete this study. She has been supportive in both my research and personal life. I truly appreciate the support that I have received from her in every walk of my life.

I would also like to thank my co-supervisors Dr. Ayaz Anwar, Dr. Syed Shahabuddin, and Dr. Aye Aye Mon for their support and guidance. I acknowledge the support of all staff and technicians at UPNM and Sunway University. Without their help, I could not complete my experiments. I gratefully acknowledge the privileges and opportunities offered by the University of Pertahanan Nasional Malaysia and Sunway University.

Finally, I would like to thank my beloved husband, ***Dr. Muhammad Shakeel Ahmad***. He supported me not only at households, to care for my toddler but also helped me in my work by improving my conceptualizations, motivating me, and helping me with computer software, graphics, and formatting. I will always be indebted to him.

APPROVAL

The Examination Committee has met on **28 September 2021** to conduct the final examination of **Bushra Rashid** on his degree thesis entitled **"**SURFACE MODIFICATION OF 2-D MXENE AND EVALUATION OF CYTOTOXICITY AND PHOTOTHERMAL THERAPY **.**

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LIST OF ABBREVIATIONS

FDA	---	Food and drug administration
WHO:	--	World health organization
PTT:	----	Photothermal therapy
Au:	--	Gold
Ag:	----	Silver
NPs	-----	Nanoparticles
HF	-----	Hydrofluoric acid
KF	------	Potassium Fluoride
NaF	------	Sodium Flouride
LiF	-----	Lithium Flouride
NaOH	------	Sodium Hydroxide
MoOx:	--	Molybdenum oxide
WS_2:	----	Tungsten disulfide
CuSe:	--	Copper selenide
2-D:	----	Two-dimensional
DNA	--	Deoxyribonucleic acid
DOX:	----	Doxorubicin
PEG:	--	Polyethylene glycol
PPG:	----	Polypropylene glycol
SEM:	--	Scanning electron microscopy
CML	----	Chronic Myeloid Leukemia
INF	--	Interferon
HER	----	Human Estrogen Receptor
CNTs	--	Carbon Nanotubes
HPV	----	Human Papilloma Virus

SiRNA	--	Small interfering Ribonucleic acid
SWCNTs	----	Single-walled carbon nanotubes
LNP	--	Lipid Base Nanoparticles
NIR	----	Near Infra-Red
STAT	--	Signal Transducer and Activator of Transcription Proteins
SOCS1	----	Suppressor of cytokine signalling
IL-6	--	Interleukin -6
IL-10	---	Interleukin -10
ROS	--	Reactive Oxygen Species
SIPGP	---	Self-initiated photo grafting and polymerization
UV	--	Ultraviolet
CT	---	Computed Tomography
PA	----	Photo Acoustic
PDT	--	Photo Dynamic Therapy
XRD	--	X-Ray Diffraction
FTIR	----	Fourier Transform Infrared Spectroscopy
SEI	--	Secondary Electron Imaging
MCF-7 cell	----	Human breast cancer cell line(Michigan Cancer Foundation)-7
A375 cell	--	Human malignant Melanoma cell
HaCaT cell	----	Human immortalized keratinocytes cell line
MCF-10A	--	Human normal mammary epithelial cells
DI	----	Deionized water

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is the second major cause of death worldwide claiming 8.97 million deaths per year and is likely to become the first in 2060 with nearly 18.63 million deaths yearly (Mattiuzzi & Lippi, 2019). It is predicted that in the coming decades, Asia will be the major prey for cancer diseases and will have more than fifty percent of cancer-related global deaths. In Malaysia, cancer is the third or fourth leading cause of medically certified deaths (Bray et al., 2018). Cancer incidence reported in Malaysia is 30,000 cases per year and its incidence is expected to rise with an increase in the aging population (Lim, 2002). Cancer is having an increasingly deleterious effect on the world's economy. Approximately $1.16 trillion was the global yearly cost in 2010 for cancer only (Stewart & Kleihues, 2003). In 2015, the United States spent $183 billion on cancer-related health services, and this figure will rise to $246 billion by 2030(*The Costs of Cancer*, 2020) and in Brazil may reach approximately INT$ 81 billion in the year 2020 (Siqueira et al., 2017). Early detection of the pre-cancerous and early-stage cancers, adequate and effective treatment may reduce cancer-related mortality and morbidity. Investing 11 billion dollars in cancer prevention programs in low- and middle-income countries could save 100 billion dollars in cancer care costs (Knaul et al., 2012).

The treatment modalities available for malignancy are surgical, chemotherapy, radiotherapy, chemo radiotherapy, immunotherapy, hormone therapy, and targeted drug therapy (Wang et al., 2018). Unfortunately, every modality has its limitations and side effects. So, there is an urgent need to find out an effective and safe approach that may increase life expectancy with improving health quality.

Medical science has been revolutionized by nano technologies. This has introduced the *Theranostic modality* in oncology, by which it is possible to screen the tumour areas and deliver the drug at the targeted sites at the same time (Vlad et al., 2015). The nano particles used, exhibit unique biological, chemical, and physical properties that selectively exhibit the potential to increase the treatment efficacy and limit the side effects on healthy tissues, ultimately leading to increased survival rates of cancer patients(Jurj et al., 2017). The major advantage of nano materials is their tunable properties, easy surface functionalization, high surface area, and tunable size (Goldberg, 2019). Nano-medicine provides a flexible network of biocompatible and biodegradable systems capable of delivering *in-vivo* traditional chemotherapeutic drugs, increasing their bioavailability and tumour tissue concentration, and enhancing their release profile thus will have various uses, ranging from diagnosis to therapy, nanoparticles can be exploited (Pucci et al.).

Various nanoparticles i.e., gold (Au), silver (Ag), carbon nanotubes (Wei et al., 2019), grapheme (Wei et al., 2019), molybdenum oxide (MoOx) (Yin et al., 2018), tungsten disulfide (WS_2) (Wei et al., 2019), copper selenide (CuSe) (Zhen et al., 2018), self-assembled organic polyamic materials (Guo et al., 2019) and dyes have been successfully tried along with their combinations and shapes with promising cytotoxicity towards normal and cancerous cells and high photo response in the

biocompatible infrared region. Recently, low energy photothermal therapy (PTT) to fight against cancer gained immense scientific interest due to its utilization of low energy radiations (visible to the infrared range) and effective hyperthermic effect. The principle of PTT is the production of heat energy near tumour cells. The heating effect would successfully kill the cancer cells without harming the normal cells as cancerous cells are more sensitive to high temperatures as compared to healthy cells(Wei et al., 2019). The ideal PTT agent should be photo-responsive and harmless for healthy cells and the affected areas can be selectively targeted through laser irradiation (Khot et al., 2019). Other than photothermal effect, the synergistic effect of PTT agents as drug carriers has also been investigated with reasonable success (Yang et al., 2019).

Recently, a new class of materials, two-dimensional (2-D) transition metal carbides/nitrides (MXenes) have been widely investigated in the biomedical field due to their biocompatibility, wide surface area, and high photo response. The MXenes ("Mn+1XnTx", X is for nitrogen and /or carbon M is any 'transition metal", and T can be any surface functional group. The 2-D sheet-like structures are obtained from selective leaching of the A-group element from its most common precursor in MAX phase ceramic material by using strong acid like hydrofluoric acid (Szuplewska et al., 2020). Bare MXenes suffer the problem of re-stacking of the nanosheets which reduces the surface area and so do its active sites and overall stability and efficiency. But MXenes have multiple binding sites for surface modification and MXenes can bind with a variety of molecules thus exhibiting a variety of properties as well as enhancing the efficiency and stability after surface modification.(Bu et al., 2020).

In a study by Jastrzębska et al., the cytotoxicity of MXenes (Ti_3C_2) has been investigated against normal and cancerous cells. The results revealed high cytotoxicity

towards cancerous cells and a lower effect on normal cells under room temperature without external stimulation (Jastrzębska et al., 2017).

In a study by Liu, et. al., Ti_3C_2 has been surface modified with doxorubicin (DOX) for enhanced effectiveness and photoactive drug release on the target area (G. Liu et al., 2017). In another study by H Lin, et al, Ti_3C_2 modified with soybean phospholipids (Lin, Wang, et al., 2017) and polyethylene glycol (PEG) also showed promising results as anticancer agents.

Although the surface-modified MXenes showed promising results as anti-cancer agents they could also be relatively more toxic to normal cells and very scanty literature is available in this regard. According to Szuplewskaet al., Ti_2C PEGylated MXene showed increased cytotoxicity towards cancerous cells and to normal cells as well at higher concentrations as compared to bare MXenes when tested against A375 (human skin malignant melanoma cell), HaCaT (human immortalized keratinocyte), MCF-7 (human breast cancer cells) and MCF-10A (normal human mammary epithelial cells) cell lines to gradually increasing concentrations (0–500 mg L^{-1}) of the studied material (Szuplewska et al., 2019). Moreover, depending on the manufacturing process, PEGs may include detectable quantities of toxic compounds like ethylene oxide and 1,4-dioxane (Black et al., 2001). So, an attempt should be made to find relatively safer MXenes *via* different surface modifications.

In this study, for the first time, we attempted to synthesize and investigate the cytotoxicity of polypropylene glycol (PPG) surface modified Ti_3C_2 MXene against normal and malignant cells and compared it with PEGylated and Bare MXene. The photo thermal effect of modified MXenes has also been studied and compared with Bare MXene using 808 nm laser light.

1.2 Problem Statement

With the advancements in nanomedicine, the biomedical and anticancer properties of MXenes are gaining increasing interest due to their high biomedical activity, less bio-toxicity, and photo-responsive nature. But, Bare MXenes suffer the problem of re-stacking of the nanosheets and poor dispersion stability in physiological media (Szuplewska et al., 2019). This greatly reduces the opportunity to harness the maximum anticancer potential of MXenes. Surface modification of MXenes is the way forward in preventing re-stacking of the MXenes and enhancing its anticancer activity. Poly ethylene glycol (PEG) surface modified MXene has been reported to enhance anticancer potential, but depending on the manufacturing process, PEGs may include detectable quantities of ethylene oxide a known human carcinogen and 1,4-dioxane a possible human carcinogen (Black et al., 2001) and severe allergic reaction have been reported with PEG (Abrams & Vander Leek, 2021; Cabanillas et al., 2020). PEG surface modified MXenes were also reported to be showing increased toxicity in normal cells when compared to bare MXenes (Han, Huang, et al., 2018). In this regard, polypropylene glycol (PPG) could be a good candidate to surface modify the MXenes on the basis that it photodegrades less compared to PEG and is also a safe food additive (Lake, 1993). Therefore, in this study, for the first time, an attempt to surface modify the 2D Ti_3C_2-based MXene with PPG via wet chemical etching was done. The resultant nanocomposite was investigated for its *in vitro* cytotoxicity and photo thermal efficiency against cancerous (A375) and normal (HaCaT) skin cells as well as on malignant (MCF-7) and non-malignant (MCF-10A) breast cell lines.