RGC Ref. No.:
UGC/FDS17/M06/19
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines: *1. Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
*2. Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

To evaluate ALA-based Photodynamic Therapy efficacy for sex-hormone dependent gynecological cancers using a new in vitro cell culture model

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Prof. CHU Shihng-meir, Professor	School of Medical and Health Sciences / Tung Wah College
Co-Investigator(s)	Prof. HUANG Zheng, Professor & Director	Biomedical Photonics Center, MOE Key Laboratory of Photonics Science and Technology for Medicine, School of OptoElectronic and Information Engineering, Fujian Normal University, Fuzhou, China
Co-Investigator(s)	Dr. WU Wing-kei, Lecturer	Department of Biological and Biomedical Sciences, School of Health and Life Sciences, Glasgow Caledonian University, United Kingdom
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2020	NA	NA
Project Completion Date	30/06/2022	31/12/2022	18/05/2022
Duration *(in month)*	30	36	18/05/2022
Deadline for Submission of Completion Report	30/06/2023	31/12/2023	18/05/2022

4.3 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

NA

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

This study aims to:

1. develop a medium exchange culture model for the hormonal study of sex hormone-dependent cancer cells;
2. determine the effects of fluctuating E2 and P levels on H-ALA-induced PpIX accumulation and localization, and the efficacy of H-ALA-PDT in cancer cells using flow cytometry, confocal microscopy and the new culture model developed in Objective 1;
3. elucidate the molecular mechanisms by which E2 and P levels modulate the rate-determining enzymes involved in H-ALA-induced PpIX production at the transcriptional and functional levels using ELISA and flow cytometric analysis, respectively, in the proposed cancer cells; and
4. generate scientific evidence for the effects of E2 and P levels on the efficacy of ALA-PDT in sex hormone-dependent cancer cell models to bridge the gap between *in vitro* and *in vivo* studies, and thus improve and enhance ALA-PDT treatment for sex hormone-dependent cancers.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change: NA

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Objective 1 was achieved. The medium exchange culture model was developed. It has been tested and evaluated the medium exchange protocol with the time-controlled release of 17β-estradiol (E2) and progesterone (PG) to the medium and the protocol was optimized for the cell culture of the proposed cell lines.

Objective 2 was achieved. The proposed cell lines were cultured in the developed model for the determination of hexyl-ALA mediated protoporphyrin IX (PpIX) generation and the intracellular localization of PpIX in the proposed cells compared with the cells that cultured in conventional cell culture model. The PpIX generation and the intracellular localization of PpIX were determined by flow cytometry and with the molecular probes by confocal microscopy respectively. The PpIX generated was higher in the hormonal dependent cells that cultured in the developed model but not in hormonal independent cells, indicating the two hormones might play a role in the heme biosynthetic pathway that generated PpIX. The PpIX was localized in the mitochondria which was independent to the presence of hormones.

Objective 3 was achieved. The three major rate-determining enzymes for the generation of PpIX in the heme biosynthetic pathway, namely CPOX, PPOX and FECH, were determined at transcription and functional levels in the proposed cancer cells that cultured in the developed model and compared to the cells that cultured in conventional condition. Results demonstrated that the expression of the heme enzymes were modulated via the enhancement of the efficacy of ALA-based PDT in the hormonal microenvironment.

Objective 4 was achieved. The developed culture model provided a simulated hormonal microenvironment as in normal human body for the hormonal dependent cancer cell growth and for the evaluation of ALA-PDT treatment efficacy. The results generated in this study could bridging the gap between *in vitro* and *in vivo* studies especially for the hormonal-dependent cancers, thus providing insights for future clinical development of ALA-PDT for hormonal dependent cancers.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. develop a medium exchange culture model for the hormonal study of sex hormone- dependent cancer cells;	√	100%
2. determine the effects of fluctuating E2 and P levels on H-ALA-induced PpIX accumu- lation and localisation, and the efficacy of H-ALA-PDT in cancer cells using flow cytometry, confocal microscopy & the new culture model developed in objective 1	√	100%

3. elucidate the molecular mechanisms by which E2 and P levels modulate the rate-determining enzymes involved in H-ALA-induced PpIX production at the transcriptional and functional levels using ELISA and flow cytometric analysis, respective- ly, in the proposed cancer cells	√	100%
4. generate scientific evidence for the effects of E2 and P levels on the efficacy of ALA-PDT in sex hormone-dependent cancer cell models to bridge the gap between *in vitro* and *in vivo* studies, and thus improve and enhance ALA-PDT treatment for sex hormone-dependent cancers	√	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

The major findings of this project can be summarized: (1) the development of the medium exchange system with the optimized protocol to provide a simulated hormonal microenvironment for cancer cell culture; (2) the efficacy of ALA-PDT was enhanced especially in the hormonal dependent cancer cells instead of hormonal independent cancer cells when cultured in the developed system; (3) the enhanced PDT efficacy was modulated by the rate-determining enzymes in the heme biosynthetic pathway.

(1) Development of the medium exchange system and the optimized culture conditions
The medium exchange system was developed with the tailor-made settings to control the exchange of hormonal medium in order to provide the hormonal microenvironment that mimic the normal human physiological changes for cancer cell growth. Such conditions were optimized and controlled for the cancer cell growth.

(2) Efficacy of ALA-PDT on the proposed cancer cells that cultured by the system
Results demonstrated that the PpIX generated from Hexyl-ALA was increased in the hormonal dependent cancer cells that cultured by the system when compared to the hormonal independent cells and the cells that cultured using conventional conditions. Besides, the intracellular localization of PpIX was in the mitochondria which in lined with the previous findings. The phototoxicity of ALA-PDT was in dose-dependent manner, in which enhanced by 18 to 40% especially in the hormonal dependent cancer cells that cultured by the system.

(3) Enhancement of PDT efficacy by the rate-determining enzymes via the heme pathway
The three rate-determining enzymes namely CPOX, PPOX and FECH were up-regulated by Hexyl-ALA-PDT in the uterine sarcoma cells cultured in the system and only PPOX was significantly increased at functional level. Hexyl-ALA-PDT also triggered significant increase of CPOX and PPOX expressions in breast cancer cells that cultured by the system.

The above results generated were published in four journal supplements and presented in six international conferences as conference abstracts. An additional journal publication is expected as invited by the chief editor of the special issue of the journal of Photodiagnosis and Photodynamic Therapy (PDPDT) for submission and waiting for the outcome.

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

Based on the results generated from this study, there were two possible research directions could further be investigated:

The mechanistic interactions and targets of the rate-determining enzymes, hexyl-ALA-PDT and the hormonal microenvironment in hormonal dependent cancers can be investigated in order to study the hormonal specific responses and to evaluate the modulated targets of hexyl-ALA-PDT by the hormones in hormonal dependent cancer cells. In addition, such investigation can further be extended to other cancers.

To culture the cancer cells in the form of organoids using the developed system, as most solid cancers grow in form of 3D structure or organoid-like structure. In order to explicit the in-depth mechanism of PDT for clinical settings, use of organoids to study the cellular response to PDT treatment especially those being cultured in a simulated hormonal microenvironment, in which the cellular responses might be more comparable to those responses inside human body, thus bridging the gap between *in vitro* to *in vivo* studies.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

The conventional *in vitro* cancer cell culture model only provided limited information for treatment regime. This study developed a medium exchange cell culture model with optimized protocol that mimic the hormonal microenvironment as in human body for cancer cell growth for the study of Photodynamic Therapy (PDT) efficacy. The results indicated that the PDT efficacy was enhanced to 18% to 40% in the cancer cells that cultured by the developed system compared to that cultured in conventional conditions. The rate-determining enzymes in the heme pathway were involved and their expression-mediated by PDT was modulated in the cancer cells that cultured by the system. All these findings provide new insights that the developed cell culture system is more suitable for *in vitro* investigation on PDT effect especially for hormonal dependent cancers.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project
(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
Nov 2020				**Chu ESM*,** Wu RWK, Huang Z.	The effect of progesterone on ALA-based PDT efficacy in uterine sarcoma cells. Annals of Oncology, Volume 31, Supplement 6, Nov 2020, Page S1339 (https://doi.org/10.1016/j.annonc.2020.10.242)	**Yes**	**Yes (Attachment 1a)**	**Yes**	**Yes**
July 2021				**Chu ESM*,** Au CM, Chau NK, Wong TS, Wu RWK, Huang Z.	An in vitro study of ALA-based PDT efficacy on uterine sarcoma in hormonal supplemented microenvironment. Annals of Oncology, Volume 32, Supplement 4, 2021, Page S339 (https://doi.org/10.1016/j.annonc.2021.05.708)	**No**	**Yes (Attachment 1b)**	**Yes**	**Yes**

Oct 2021				**Chu ESM*,** Wu RWK, Lau HY, Huang Z.	Enhanced 5-aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) efficacy by hormones in uterine sarcoma cells via upregulation of proporphyrinogen oxidase. Annals of Oncology, Volume 32, Supplement 6, 2021, Page S1359. (https://doi.org/10.1016/j.annonc.2021.08.2042)	**No**	**Yes (Attachment 1c)**	**Yes**	**Yes**
July 2022				**Chu ESM*,** Wu RWK, Lau HY, Huang Z.	To establish a simulated hormonal microenvironment culture model for advancement of PDT modality in gynecological cancer. Annals of Oncology, Vol 33, Supplement S6, 2022, Page S492-S493 (https://doi.org/10.1016/j.annonc.2022.05.144)	**No**	**Yes (Attachment 1d)**	**Yes**	**Yes**
Dec 2022				Leung G, Cheung K, Wu R, Huang Z, **Chu ESM***	Hormonal modulation of photodynamic therapy efficacy in breast cancer 3D spheroid	**No**	**Yes (Attachment 1e)**	**Yes**	**Yes**

					culture model. Annals of Oncology, 33 (suppl 9): S1444 (https://doi.org/10.1016/j.annonc.2022.10.048)				
2024		√ **(Manuscript no.: PDPDT-D-23-01086)**		**Chu ESM*,** Wu RWK, Huang Z.	Potential therapeutic efficacy of Photodynamic Therapy on female hormonal-dependent cancers in a hormonal simulated microenvironment. Photodiagnosis and Photodynamic Therapy (PDPDT)	**No**	**Yes (Attachment 2)**	**Yes**	**Yes**

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Nov/2020/ Singapore	The effect of progesterone on ALA-based PDT efficacy in uterine sarcoma cells	European Society of Medical Oncology (ESMO) Asia Virtual Congress 2020	Yes (progress report in Feb 2021)	Yes **(Attachment 1a)**	Yes	
Mar/2021/ Japan	An in vitro study of ALA-based PDT efficacy on uterine sarcoma in hormonal supplemented microenvironment	Japanese Society of Medical Oncology (JSMO) 2021 Virtual Congress	Yes (progress report in Feb 2021)	Yes **(Attachment 1b)**	Yes	

Oct/2021	Enhanced 5-aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) efficacy by hormones in uterine sarcoma cells via upregulation of proporphyrinogen oxidase	Molecular Analysis for Precision (MAP) Oncology Virtual Congress 2021	No	Yes **(Attachment 1c)**	Yes	
Feb/2022/ Japan	To establish a simulated hormonal microenvironment culture model for advancement of PDT modality in gynecological cancer	Japanese Society of Medical Oncology (JSMO) 2023	No	Yes **(Attachment 1d)**	Yes	
Dec/2022/ Singapore	Hormonal modulation of photodynamic therapy efficacy in breast cancer 3D spheroid culture model	European Society of Medical Oncology (ESMO) Asia Congress 2022	No	Yes **(Attachment 1e)**	Yes	
Jul/2023/ Finland	Potential therapeutic efficacy of Photodynamic Therapy on triple negative breast cancer in hormonal microenvironment	International Photodynamic Therapy (IPA) 2023	No	Yes **(Attachment 1f)**	Yes	

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

There were 5 undergraduate students participated in this project as the final year project. Three of them worked as a group during the period of Jan to June 2020. The results generated by these three students were presented in an international conference in Mar 2021 and with a publication of a journal supplement. The data generated by another two students were presented in two international conferences in Dec 2022 and July 2023. The two students also joined the conference for poster presentation accordingly. Their presentations were shared to over 200 international participants during the conferences in Singapore and Finland respectively. And a journal supplement was also published in Dec 2022.

The students' final year report also presented to their peers of 30 and 45 classmates in their cohort of study in June 2020 and June 2022 respectively.

A workshop on cell culture techniques was arranged for the students who attended the course of Principles of Molecular Diagnostics and Final Honours Project in May 2022.

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	**Degree Registered for**	**Date of Registration**	**Date of Thesis Submission / Graduation**
[redacted]	Bachelor of Medical Science (Honours) (Major in Medical Laboratory Science)	September 2016	June 2020 / Nov 2020

[redacted]	Bachelor of Medical Science (Honours) (Major in Medical Laboratory Science)	September 2016	June 2020 / Nov 2020
[redacted]	Bachelor of Medical Science (Honours) (Major in Medical Laboratory Science)	September 2016	June 2020 / Nov 2020
[redacted]	Bachelor of Science (Honours) in Medical Laboratory Science	September 2018	June 2022 / Nov 2022
[redacted]	Bachelor of Science (Honours) in Medical Laboratory Science	September 2018	June 2022 / Nov 2022

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

The PI received two Travel Grant Awards by the Japanese Society of Medical Oncology (JSMO) in 2022 and 2023.

Further strengthen research collaboration with Fujian Normal University, Fuzhou, China and the Department of Biological and Biomedical Sciences, School of Health and Life Sciences, Glasgow Caledonian University, United Kingdom.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	**Conference Papers**	**Scholarly Books, Monographs and Chapters**	**Patents Awarded**	**Other Research Outputs (please specify)**	
No. of outputs arising directly from this research project	1 (under review)	6	NA	NA	Type	No.
					Student Final year project	5
					Journal supplement	5
					Travel Award	2

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	**Reasons**
NIL	