RGC Ref. No.:
UGC/FDS17/M02/15
(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

Investigate FosPeg® mediated PDT efficiency on Nasopharyngeal Carcinoma using 3D cell model approaches

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. WU Wing-kei, Assistant Professor	School of Medical and Health Sciences, Tung Wah College
Co-Investigator(s)	Dr. CHU Shihng-meir, Assistant Professor	School of Medical and Health Sciences, Tung Wah College
	Dr. YUEN Wai Man, Associate Professor	School of Nursing, The Hong Kong Polytechnic University
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2016	N/A	N/A
Project Completion Date	31/12/2017	31/12/2018	04/10/2018
Duration *(in month)*	24	36	N/A

Deadline for Submission of Completion Report	31/12/2018	31/12/2019	N/A

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To develop an *in vitro* 3D cell culture model using NPC cell line C666-1.
2. To image the uptake and localization of FosPeg® using the cell model established in objective 1.
3. To optimize the dosimetry of FosPeg® mediated PDT using the cell model established in objective 1.
4. To investigate the *in vitro* effects of FosPeg® mediated PDT on photobleaching, mode of cell death, cell migration and modulation of intracellular signaling pathways (MMPs and ERK) and alternation of drug resistance protein expression (P-gp) using the cell model established in objective 1.
5. To enhance research capacity of PI, Co-Is and students in Department of Medical Science and transfer the research experiences and new knowledge into teaching and learning.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change:

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. Two *in vitro* 3D culture models were established using NPC C666-1 cells by the liquid overlay method with agarose base (MCL) and the hanging drop method (MCS). The 3D spheroid established by MCL method was more compact, rigid and spherically shaped, with an average diameter of 539.6 ± 29.9 μm at day 3 culture. In contrast, the 3D spheroid established by MCS method was more irregular and less compact, with an average diameter of 683.1 ± 82.2 μm at day 3. The gene expression in 2D and 3D models were compared and results showed a significant reduction of LMP1 mRNA and ABCG2 mRNA expression in MCL spheroids in compare with the 2D cells.

Objective 2 was achieved. The 3D models established were used to study the uptake and distribution of photosensitizer (PS) FosPeg® via flow cytometry and confocal microscopy. FosPeg® accumulated in 2D cells and 3D spheroids in similar uptake profiles. No significant difference of FosPeg® accumulation was found between MCL and MCS spheroids but it was significantly higher in 2D cells. The distribution of PS in 3D spheroids were also evaluated. PS were also found mainly accumulate in the external cell rim of the MCL and MCS spheroids at 24 h when incubated with 1 μg/mL FosPeg®.

Objective 3 was achieved. FosPeg® mediated PDT were effective to kill NPC cells in 2D and 3D models. For 2D cells, IC_{25} and IC_{50} were achieved at 0.001 μg/mL and 0.0025 μg/mL FosPeg® with 20 J/cm^2 of light activation respectively. For 3D MCL and MCS spheroids, IC_{25} and IC_{50} were achieved at 0.1 μg/mL and 1 μg/mL FosPeg® with 20 J/cm^2 light activation respectively. 3D spheroids were less sensitive to FosPeg® PDT as compared to 2D cells as a 400 times higher FosPeg® concentration were required to achieve IC_{50}.

Objective 4 was achieved. Photobleaching effect was observed at post 10 minutes PDT in both 2D and 3D models. FosPeg® PDT mainly triggered apoptosis in 2D cells and 3D spheroids at 24 h post PDT despites the difference in the structure pattern of the cultured cells. Our findings revealed that PDT induced LC3B protein expression in 2D and 3D models in a time dependent manner. The ratio of LC3B II/LC3B I indicated the accumulation of autophaosomes were first detected at 2 h post PDT in MCL spheroids, 4 h post PDT in MCS spheroids but at 24 h post-PDT in 2D cells, respectively. Difference in protein expression to FosPeg® PDT were observed in 2D and 3D models. LMP1 was up-regulated by PDT in both 3D spheroids in a time dependent manner but no significant difference was found in 2D cells. MMP2 and MMP9 were also up-regulated in 3D spheroids in a time dependent manner. Interestingly, the response of 2D cells was different as the reduction of MMP2 and MMP9 were observed at 24 h post-PDT in 2D cells. Up-regulation of ABCB1 and ABCC1 expression were also observed in 3D spheroids in a time dependent manner. However, the ABCB1 protein expression was reduced at 24 h post-PDT and the ABCC1 protein expression remain unchanged in 2D cells.

All these findings indicate that 3D spheroids, especially the MCL spheroids, are more suitable for *in vitro* evaluation of PDT effect.

Objective 5 was achieved.

The scientific evidence collected from this study allows the PI and Co-Is to have a better understanding in the relationship between PDT effect and 3D culture models. Moreover, the 3D models established will continuously be used by the PI and Co-Is for future research grant applications.

The research experience and new knowledge generated by this project were transformed into teaching and learning. Part of the knowledge generated by this project was delivered in the courses MED1003 Molecules, Cells and Genes and MED3011 Human Genetics. The 3D model established in this study has been used in the undergraduate students' final year projects and will be continuously used in future.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To develop an *in vitro* 3D cell culture model using NPC cell line C666-1.	√	100%
2. To image the uptake and localization of FosPeg® using the cell model established in objective 1.	√	100%
3. To optimize the dosimetry of FosPeg®-mediated PDT using the cell model established in objective 1.	√	100%
4. To investigate the *in vitro* effects of FosPeg®-mediated PDT on photobleaching, mode of cell death, cell migration and modulation of intracellular signaling pathways (MMPs and ERK) and alternation of drug resistance protein expression (P-gp) using the cell model established in objective 1.	√	100%
5. To enhance research capacity of PI, Co-Is and students in Department of Medical Science and transfer the research experiences and new knowledge into teaching and learning.	√	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 study have partially published in two publications. Two *in vitro* 3D culture models were established using NPC C666-1 cells by the liquid overlay method with agarose base (MCL) and the hanging drop method (MCS) and the gene expression in 2D and 3D NPC models were compared at the first time. A lower LMP1 mRNA and ABCG2 mRNA expression was found in MCL spheroids in compare with the 2D cells, indicated that cells in MCL spheroid might be transformed and proliferated rapidly and might have stable EBV genome retention.

By using the 3D models established, the uptake of photosensitizer (PS) FosPeg® were measured. No significant difference of FosPeg® accumulation was found between MCL and MCS spheroids but it was significantly higher in 2D cells. It was 2.6 times higher in 2D cells at 24 h of incubation. In this relation, at the tested PS dosage range, 3D spheroids showed less sensitive to FosPeg® PDT as compared to 2D cells as a 400 times higher FosPeg® concentration with 20 J/cm^2 light activation were required to achieve IC_{50} (WU et al., 2017). The FosPeg® PDT mediated apoptosis/necrosis were analyzed and results indicated that FosPeg® PDT mainly triggered apoptosis in 2D cells and 3D spheroids despites the difference in the structure pattern of the cultured cells, as a similar distribution of apoptotic cells and necrotic cells were observed at 24 h post PDT (WU et al., 2018). Additionally, further analysis of the protein expression indicated that 2D and 3D models response differently to FosPeg® PDT. The PDT induced up-regulation of LMP1, MMP2 and MMP9 proteins in 3D models but not in 2D models showed that cells might behaved differently in 2D and 3D models. All these findings suggested that 3D spheroids, especially the MCL spheroids, are more suitable for *in vitro* evaluation of PDT effect.

An additional journal publication is expected in the journal of photochemistry photobiology: Biology.

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

This is the first study to show different LMP1 gene expression profiles in 2D and 3D NPC cell models. Moreover, 3D spheroids responded differently to PDT from 2D cells. All these findings indicate that 3D spheroids, especially the MCL spheroids, are more suitable for *in vitro* evaluation of PDT effect. The 3D spheroids established in this study provides the PI a better *in vitro* model to evaluate the relationship between PDT, the hypoxia conditions in tumor cell mass, immune response, cell-to-cell communication, and NPC/EBV associated molecular changes.

The relationship between PDT and the hypoxia conditions in MCL 3D spheroids can be measured by the chemical agents dihydro-rhodamine 123 (DHR123)/rhodamine 123 (R123). The relationship between PDT, immunotherapy and immune checkpoint blockade therapy can be measured by MTT assay and qPCR. The difference in PDT mediated molecular changes in NPC 3D spheroids can be measured by the qPCR via studying the NPC and EBV related mRNAs and miRNAs, includes LMP2A, EBNA1, BART 1-5p and BART 16. The clinical relevance of MCL 3D spheroids can be studied via the comparative analysis of different models included *in vitro* 3D spheroids, *ex vivo* organ culture with 3D spheroids and *in vivo* animal test.

7. Layman's Summary

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

Photodynamic Therapy (PDT) effect depends on the photosensitizer, light and oxygen. In this *in vitro* study, two 3-dimensional (3D) culture models were established using nasopharyngeal carcinoma (NPC) cells by the liquid overlay method with agarose base (MCL) and the hanging drop method (MCS). The MCL method established spheroids with more spheroidal in shape and with a sharp outer boundary, while the MCS method established spheroids with diverse shape and with a loose outer boundary.

The gene expression profile in 2D and 3D models was compared at the first time. The expression level of NPC specific oncogene LMP1 was lower in MCL spheroid than in 2D cells. The sensitizer uptake in 3D spheroids was half of 2D culture at 24 hours incubation. PDT were effective to both 2D and 3D models, but more sensitizer were required to achieve inhibitory concentration (IC_{50}) in 3D spheroids than in 2D cells. Apoptosis, necrosis and autophagosomes were detected in PDT treated 2D and 3D cells. Different protein expression patterns were observed in 2D and 3D models. All these findings indicate that 3D spheroids, especially the MCL spheroids, are more suitable for *in vitro* evaluation of PDT effect.

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 correspond-ing 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)*	**Acknowl-edged 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)*						
2017				Wu RWK*, Chu ESM, Yuen JWM, Huang Z	Investigate the efficacy of Photodynamic Therapy on nasopharyngeal carcinoma multicellular tumor spheroids. Annals of Oncology, Volume 28, Issue suppl_10, November 2017, mdx652.012.	**No**	**Yes**	**Yes**	**Yes**
2018				Wu RWK*, Chu ESM, Yuen JWM, Huang Z	Photodynamic therapy induced apoptosis and autophagy in 2D and 3D nasopharyngeal carcinoma cell models. Annals of Oncology,	**No**	**Yes**	**Yes**	**Yes**

					Volume 29, Issue suppl_7, October 2018, mdy375.078.				
2019				Wu RWK*, Chu ESM, Yuen JWM, Huang Z	Difference of PDT induced apoptotic gene expression in 2D and 3D nasopharyngeal carcinoma cell models. Annals of Oncology, Volume 30, Issue Supplement_6, October 2019, mdz343.074.	**No**	**Yes**	**Yes**	**Yes**
		2019		Wu RWK*, Chu ESM, Yuen JWM, Huang Z	Comparative study of FosPeg® photodynamic effect on nasopharyngeal carcinoma cells in 2D and 3D models. Journal of Photochelistry and Photobiology B:Biology.	**No**	**Yes**	**Yes**	**No**

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)*
Jun / 2017 / Seoul, Korea	Evaluate the effect of photodynamic therapy using 3D nasopharyngeal carcinoma cell culture models.	24th Asia Pacific Cancer Conference in conjunction with the 43rd Annual Meeting of Korea Cancer Association.	Yes, 2017 annual report	Yes	Yes	Yes
Nov / 2017 / Singapore	Investigate the efficacy of Photodynamic Therapy on nasopharyngeal carcinoma multicellular tumor spheroids.	ESMO Asia 2017 congress.	Yes, 2017 annual report	Yes	Yes	Yes
Jun / 2018 / Seoul, Korea	Modulation of MMP-2 expression by Photodynamic therapy in 2D monolayer culture and 3D spheroid culture models. (Poster award)	The 44th Annual Meeting of Korean Cancer Association with the 4th International Cancer Conference. (**Poster award**)	No	Yes	Yes	Yes
Jul / 2018 / Kobe, Japan	Photodynamic therapy induced apoptosis and autophagy in 2D and 3D nasopharyngeal carcinoma cell models.	2018 Japanese Society of Medical Oncology Annual Meeting.	No	Yes	Yes	Yes
Nov / 2018 / Tokyo, Japan	Study of the PDT effectiveness on nasopharyngeal carcinoma using different cell culture models. (Invited speaker)	2018 LaserWeek in Tokyo. (**Invited speaker**)	No	Yes	Yes	Yes

Jul / 2019 / Kyoto, Japan	Difference of PDT induced apoptotic gene expression in 2D and 3D nasopharyngeal carcinoma cell models.	2019 Japanese Society of Medical Oncology Annual Meeting.	No	Yes	Yes	Yes

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

(Please elaborate)

Tung Wah College is a self-financing institution offering programmes at Higher diploma and Bachelor degree level. The research experience and new knowledge gained in this study has been transferred mainly in the classroom teaching and in the final year project training.

The research experience obtained in this study has been transferred to teaching and learning as: 1) two student helpers were recruited in this project, 2) the 3D models established has been used in the final year projects. Student helpers and project students were not only required to carry out laboratory work but also required to attend laboratory meetings in order to train up their research capacity. Findings obtained from student project has been presented in the international conference (The 44th Annual Meeting of Korean Cancer Association with the 4th International Cancer Conference). The 3D models established will also be continuously used for student projects.

The new knowledge has also been transferred to teaching and learning. New knowledge related to tumor growth in 3D cultures and anti-tumor agents induced mode of cell death and molecular changes in 3D cultures have been delivered in the courses MED1003 Molecules, Cells and Genes and MED3011 Human Genetics.

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
	Bachelor of Medical Science (Forensic Science major)	09-2014	05-2018 / 10 - 2018
	Bachelor of Medical Science (Basic Medical Science major)	09-2014	05-2018 / 10 - 2018

12. Other Impact

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

The PI received a poster award in the 44th Annual Meeting of Korean Cancer Association with the 4th International Cancer Conference and had been invited as a speaker in the 2018 LaserWeek in Tokyo conference.

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	3	6	0	0	Type	No.
					Poster award	1

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	