RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: **15 September 2011**
Project completion date: **14 September 2016**

1. Project Title: Cell Based Heart Regeneration

2. Names and Academic Affiliations of Project Team Members#

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator (PC)**	Ronald A. Li	Li Dak Sum Centre, HKU.	12 hrs, decreased in 5th year due to diminished role of PC
Co-Principal Investigator(s)	Kenneth R. Boheler/Professor, Deputy Director, SCRMC Deputy Project Coordinator	School of Biomedical Sciences (SBMC)/ Faculty of Medicine/ HKU	24 hrs, increase due to assumption of Deputy PC duties
	Barbara Chan/Associate Professor	Mechanical Engineering/ HKU	<1 hrs, completed project component in Year 4
	Camie Chan/Assistant	SBMS, HKU	<2 hrs, decreased during 5th year as

	Professor		no longer at HKU
	Godfrey CF Chan/Head and Professor	Paediatric & Adolescent Medicine/ HKU	4 hrs
	Shuk Han Cheng/Professor	Biology and Chemistry, CityU	8hrs
	Kenneth R. Chien/ Director & Professor	Cardiac Initiative, Karolinska Institutet/ Harvard/ HKU	<1hr
	Kevin Costa/Associate Professor	Cardiology/MSSM/HKU	<1 hr
	Roger J. Hajjar/Director & Professor	Cardiovas Research Centre/ MSSM	<1hr
	*Hon Cheung Lee/ Head & Chair Professor	Physiology/HKU	Retired from HKU
	Si Lok/Professional Consultant	Dept of Chemical Pathology & Li Ka Shing Institute of Health Sciences/ CUHK	Resigned from CUHK
	Andrew L. Miller/ Professor**	Division of Life Science/ HKUST	6hrs
	Jianan Qu/ Professor**	Engineering/ HKUST	2hrs
	Dong Sun/Professor	Mechanical and Biomedical Engineering/ CityU	6hrs
	Joseph C. Wu/Professor	Stem Cell Institute/ Stanford	< 1hr
	Hong Yan/Chair Professor	Computer Engineering/ CityU	0 hr Inactive
	Xiaoqiang Yao/Professor	School of Biomedical Sciences/ CUHK	8hrs
	*Jianbo Yue/ Assistant Professor	Physiology/HKU Now at CityU	2 hrs, as is now collaborating with H Cheng at CityU
Co-Investigator(s)	Fadi Akar/ Assistant Professor	Cardiovas. Research Center/MSSM	<1 hr
	Martin Bootman/ Project Leader	Laboratory of Signalling & Cell Fate, Babraham Institute, Babraham, Cambridge	<1 hr
	Ying Chau/ Assistant Professor	Chemical and Biomolecular Engineering/ HKUST	<1 hr
	Heping Cheng/	Institute of Molecular	<1 hr

	Co-Director & Professor	Medicine/ PKU	
	Antony Galione/ Department	Pharmocology/ Oxford	<1 hr
	Head & Professor I-Ming Hsing/ Professor	Chemical and Biomolecular Engineering/ HKUST	3 hr
	Yun Wah Lam/ Associate Professor	Biology and Chemistry/ CityU	<1 hr, component completed
	Ning Li/ Professor	Biology/ HKUST	<1 hr
	Ray Ng/Assistant Professor ** (change of affiliation)	SBMS/ HKU	1 hr
	Alfonso Ngan/ Professor	Mechanical Engineering/ HKU	<1 hr
	Zhong Wang/ Assistant Professor	Ctr CV Res/ Harvard Stem Cell Inst.	<1 hr
	Hau San Wong/ Associate Professor	Computer Science/ CityU	<1 hr, component completed
	Hongkai Wu/ Assistant Professor	Chemistry/ HKUST	2 hrs
	Huangtian Yang/ Deputy Director & Professor	Chinese Academy of Sciences, HIS & State Key Lab of Stem Cell Biology	<1 hr
	George Yuan/ Assistant Professor	Electronic & computer Engineering, HKUST	<1 hr
Collaborators	Lei Bu/ Research Assistant Professor	SCRMC, HKU	Inactive. Now an Assistant Professor at NYU Langone Medical Centre
	Kathy Lui/ Post-doctoral Fellow	SCRMC, HKU	Dr Lui graduated and accepted a position as Assistant Prof at CUHK.

*As reported in previous reports, Prof. Hon Cheung Lee resigned from his position as Head of Physiology at HKU and retired, and is no longer on the project team. Dr. Jianbo Yue, Assistant Professor in Physiology left HKU and was inactive after Prof. Lee's retirement. He, however, relocated to CityU and worked with Shuk Han Cheng to complete one project. As the original project of Prof Lee/Dr. J Yue could no longer be completed as designed, the corresponding research

component was collectively shared by the PC, Professors Boheler and Yao. Prof. Si LOK resigned from CUHK and is no longer affiliated with the TRS. The duties of Prof Lok were assumed by Prof. KR Boheler (Dec 2015). In lieu of CD38, the scope has even expanded by the three professors to include several Ca^{2+} handling proteins (e.g., TRPV4, PLB, CSQ, NCX and SERCA). The project's scientific productivity has not suffered due to this change.

** Changes in time commitment from prior reports reflect estimated average contributions of investigators in the final year.

Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

3. Project Objectives

Summary of objectives addressed/achieved:

The TRS funded *Cell-based Heart Regeneration* has now been completed. Significant advances were made in each of the four proposed Project areas, and since the 5th year interim report, additional research has been completed, with manuscripts published, accepted or in states of revision. Only with a few exceptions (in part due to loss of personnel, changes in experimental design, negative results) were Milestones, requested by the RGC at the beginning of the 5th year, not realized; however, a number of manuscripts remain to be submitted and published. Major objectives achieved in this programme can be summarized as follows. Advances in *Engineering of Human Cardiac Muscle and Chamber* included the development of Engineered Cardiac Tissues (ECTs) using technologies shared between Hong Kong and the U.S.A. (Costa, Hajjar, Li), and new technologies/platforms or technological advancements developed in Hong Kong (BP Chan; I-M Hsing; J Qu; A Miller). Extensive basic science analyses were performed examining the mechanical and electrophysiological traits of cells and cell constructs, and an evaluation of altered environments to direct hESC-CMs towards an adult phenotype were completed, but with mixed results. A spin-off company (NovoHeart), located at the Hong Kong Science & Technology Park (HKSTP) along with the first $21M R&D project approved by the Innovation Technology Fund (ITF) was established that has provided jobs for some graduates of this TRS program and others in Hong Kong. These scientific advances and interactions with companies will facilitate further basic research endeavors and the development of commercial ventures. Specifically, new collaborations and activities involving the development of drug screening for Pharma not covered by the present TRS grant have been initiated. The *Biology of Cell Engineering* Project led to the development of novel techniques for Ca^{2+} imaging, a comprehensive analysis of TRP channels, nuclear Ca^{2+} signaling and combinatorial approaches to drive in vitro maturation of hPSC-CMs in 2D and 3D, including papers published since the last interim report. New insights regarding hypertrophy of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) and new technologies were developed to drive structural and electrophysiological development of immature cells. The mechanical modification of cells to

promote maturation (Projects 1 and 2) did not turn out as expected, but the program allowed us to apply new technologies (modified RNAs) to this problem that were not available 5 years ago. *Enhancing the Therapeutic Potential*, has seen significant advances, particularly over the final year. Professor Godfrey Chan undertook two new immunology program components that were endorsed by the SAB. As he only received funding for this starting in March of the final year, these two studies remain to be published; however, he achieved most of the proposed experimental objectives. For publication, only completion of a few experiments for the second project and final data analyses are required for submission. The surfaceome analyses contributed to the identification of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors as a mechanism for eliminating tumorigenic cells from differentiated cells destined for therapies, and it has advanced the immunophenotyping and isolation of distinct cell populations. Specifically, putative markers for chamber specificity were identified, but the monoclonal antibodies produced using public companies did not work as anticipated. A change in direction and new collaborations were thus established to generate antibodies that can be used to sort and confirm the immunostaining findings with polyclonal antibodies. In the last 6 months of the project, a novel marker of metabolic maturation was identified that may prove valuable to Pharma in the identification and use of cells with a more adult-like phenotype. This work is being completed, and also remains to be published, due to the unforeseen delays in antibody developoment. The Omic analyses have provided mechanistic insights into developmental and differentiation processes. New algorithms for decoding transcriptional regulatory networks have been developed, and in the end, this project area exceeded expectations. Advances in Pre-clinical translation moved forward; however, research with cell-based bio-sinoatrial nodes in the pig model was de-emphasized during Year 3 of the project. Through collaborations with Ken Chien at the Karolinska Institute (KI), progenitor cells have been tested for their therapeutic applications in animal models. In summary, all major goals have been completed; however, where manuscripts are not yet been fully reviewed or accepted, the percentage completed has been listed as 100% contingent upon publication thus reflecting the fact that some findings are not yet submitted or published.

i. At total of 101 peer-reviewed articles, chapters or other papers have been published. This includes 86 articles with acknowledged TRS funding in our final list (explanations provided for those lacking acknowledgement), while ~10+ manuscripts are in various stages of publication (submission, revision, in press).
ii. In Appendix **1**, we have summarized a majority of the major accomplishments achieved with TRS funding.
iii. In Appendix 2, we provide an update on the achievements related to the Milestones required by the M&A Panel, prior to 5th year funding.
iv. In Appendices 3 and 4, we provide a list of all TRS publications, and we include information from these papers showing how TRS funding was acknowledged.
v. In Appendix 5 (a file), we provide pdf files of most of the publications that acknowledged TRS funding.

vi. Since the last interim report, no additional Management Committee meetings were held; however, communication continued between the Deputy PC and the PIs to help ensure progress on the final Milestones.

vii. The output from this project has led to international recognition, the development of new collaborations and additional funding opportunities. Many of these have been described in past reports and in the interim project

viii. Given the progress that has resulted and the development of some sustained interactions during the 5th year, several PIs are actively collaborating to revise several manuscripts funded in part by the TRS, while others are taking advantage of research advances to further our scientific endeavors.

ix. A list of graduate students, and postdoctoral fellows trained as part of this TRS funded Project can be found in Section 6.3, and now includes the successful completion of one PhD student in immunology. Several of the trained individuals work with NovoHeart and the ITC funded project mentioned above or have taken other academic positions, including post-doctoral fellowships abroad.

x. Further advances have been documented in Appendix 1 and additional scientific details outlining our research progress in the form of figures and information can be found in **Appendix 2**. No additional Management Committee meeting summaries are provided, as these were present in the Interim report.

Objectives*	**Percentage achieved**	**Remarks****
Project 1, Aim 1 Biofabrication of engineered Cardiac Tissue (ECT)	100%, exceeded expectations with development of a new platform	The human ventricular (h**v**) cardiac tissue strips and organoid chambers have been developed as proposed and are now a routine procedure at HKU. We also exploited the cardiac microtissue model, which has been useful in aspects of Projects 1, 2 and 3, but it proved less robust than envisioned. The planned experiments for human cardiac organoid chambers was completed with characterization of the model as well as its implementation as a drug discovery tool that is part of the proposed tissue engineering/drug testing platform. Manuscript has been submitted and is under revision.
Project 1, Aim 2 Electrical stimulation for ECT	100%	The proposed experiments were complete by Year 4; final data collection involving T3 has been completed and 3 manuscripts drafted and submitted with another MS student graduated and a PhD student defending by Oct 2017.
Project 1, Aim 3 Modeling DMD-induced Cardiomyopathy	100%, once manuscript has been published	One manuscript was prepared for submission in Year 5. Additional data involving Trp channels were added and the manuscript has been prepared and submitted.
Project 2, Aim 1 Engineering Maturation of hESC/iPSC-derived CMs	100%	All proposed experiments were completed with publications. Additional experiments were added, since the mechanical engineering of cell maturation did not produce the desired results.

Objectives*	Percentage achieved	Remarks**
		In year 5, we studied the possibility of direct reprogramming of human fibroblast towards a cardiac fate by microinjection of synthetic mRNA. These data appear promising and an additional manuscript has been published.
Project 2 Aim 2 Role of Ca^{2+} signalling in cardiac differentiation and maturation of hESC-VCMs using the bioluminsecent Ca^{2+} reporter, apo-aequorin (HKUST)	100%	The apo-aequorin system did not prove sufficiently robust to routinely measure Ca^{2+} signals in differentiating hPSC-CMs. In Year 5, studies were initiated to determine the viability of a NanoLantern system that could prove highly informative in the future. These are additional experiments
Functional role of Ca^{2+} signalling, specifically TRP channels, in cardiac stem cells (CUHK)	100%, exceeded expectations	All experiments with TRP channels are complete. Much work has been published. Some additional manuscripts are either submitted/under revision/under preparation by Dr. Yao. This part of the grant has been highly productive.
Combinatorial approaches	100%	Utilized combinatorial approaches (T3, alignment, pacing) to drive "maturation" and enhance Ca2+ signalling. Transcriptomic analyses on the patterned monolayers and other 3D constructs undergone combinatorial treatment are now complete. Results have been added to 2 manuscripts on combinatorial approaches.
Nuclear Ca2+ signalling	100%, once all publications published	All planned studies have been completed. The movement of one PDF to the Mainland has delayed submission of two publications, but submission of these manuscripts is anticipated soon.
Project 2 Aim 3 Test if enhanced mitochondrially-derived ROS production promotes maturation	100%	Collection of metabolic data is complete, and data have been combined with combinatorial approach as in Project 2, Aim 2c. Mitochondrial data have been completed and published. Additional combinatorial approach with metabolic intervention (PPAR and fat) has been completed and submitted for publication. The manuscript is attached in the progress report

Objectives*	Percentage achieved	Remarks**
Test if energetically mature and preconditioned hPSC-CMs have improved survival and efficacy *in vivo*	(untenable, see Project 4)	Neither mechanical nor metabolic/ electrical stimuli lead to the development of energetically "mature" cells. Aspects of maturation were accomplished, but due to the experimental limits, tests of survival were deemed untenable – based on early results and failure to graft. Other studies with Progenitor cells, in collaboration with Dr. Ken Chien, have shown more promise (and as part of Project 4). A manuscript in collaboration with Chien lab has been submitted and is now under revision.
Project 3, Aim 1 Immunocompatibility for prolonged graft survival	100%, upon completion of 2 on-going projects, with publication.	We found that CMs co-cultured with dendritic cells have immune modulatory properties and work had progressed to studies in animal models. The PI responsible for this work, subsequently left HKU, and has not completed the manuscript for submission. It was turned over to the PC, and some data have been combined with the progenitor study of Project 4 to support its revision. In lieu of the setbacks associated with this project area, the Management Committee met and gave support to Prof. Godfrey Chan who is working on two other aspects of immunology. This is progressing and the SAB member who reviewed this change of direction felt that the approach was sound and that the data would lead to publications. The aim is complete for one project, pending submission and publication, while the second immunology project area is nearing experimental completion, after which time it will be submitted for publication. With publication, this project will be complete.
Project 3 Aim 2 – Identification of novel lineage-specific markers for immunophenotyping and purification of therapeutically-viable hPSC-vCMs	100%, once final papers are published	The CSC technology was successfully established, and the data have led to the identification of NAMPT inhibitors as a methods for eliminating tumorigenicity; lineage restricted and differentiation stage markers have been identified, and functional studies are being completed on the sorted cells. The chamber restricted markers required the production of new antibodies which did not go as anticipated. As the antibodies prove useless

Objectives*	Percentage achieved	Remarks**
		for Flow Cytometry, the project aims, as originally described in the 5th year Milestones, were altered to evaluate a developmental marker that could be used to identify metabolically more mature cells for disease modeling. This project has advanced well, but the research is not yet fully complete and has not yet been submitted for publication.
Project 3 Aim 3 – Systems-based analyses of human cardiac differentiation	100%, exceeded expectations	Some delays occurred due to the resignation of Si LOK from CUHK. His responsibilities were taken over by K. Boheler. Subsequent studies have progressed well, and numerous papers published. One network analysis paper was recently accepted to Nature Communications, while another one involving combinatorial regulation is being analysed beyond what was originally proposed (and we are waiting on the latest RNAseq data). A drug repurposing algorithm has been published, and it is now being incorporated into a larger algorithm for application to hPSC-CMs. This latter study, goes beyond the anticipated Milestones, allowing us to state that this project area has exceeded expectations
Project 4 Aim 1 Cell-based bio-artificial pacemaker construction	100%, once final papers are published	There were significant delays as the cells were being developed, and due to some animal model constraints. Despite this, the project is now complete. Three manuscripts have been spun out of the pacemaker study, 2 of which have already been published. Another manuscript has been submitted, but it is not yet published.
Project 4 Aim 2 Transplantation when ECTs become available	100%, once final papers are published	Two postdocs from Ken Chien's lab came to HK during the final year to wrap up a paper on myocardial repair by in vivo cardiac patch in a SCID mouse model. Parts of Project 3 data are now used to support this revision. Results show improved function with the grafting of cardiac progenitor in the MI mouse hearts. The paper has been submitted and is in the process of revision in Nature Biotechnology.

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

(Maximum 20 A4 pages for sections 6 to 9, excluding any appendices and attachments)

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.))

1) Funding of ITF Project ITS/131/13FX (leading to product IP and potential licensing); 2) Development of organic electrochemical transistors (OECTs) and use of microfluidic technologies for single cell manipulation (patents pending); 3) Improved methods for CM differentiation (many citations and international applications); 4) Development and use of ECTs, which formed the basis for the ITF funding and continued international applications (international recognition); 5) Basic science analyses of TRPV channels leading to a greater understanding and appreciation of their diverse roles in biology and particularly with emphasis on hPSC-CMs (accumulating citations and international recognition.)

6.2 What was the added value of the TRS funding, rather than standard project grant funding?

(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

This project could not have been achieved without TRS funding in large part because of the breadth of knowledge required, the need to recruit new scientists from different fields for this endeavor, and the need to share expertise. Moreover, the five-year funding period was critical, as the first 2 years required significant developments in the way of infrastructure, training of students, and methodology advancements.

6.3 If the project has not met its original objectives, why?

The Project has largely met the original goals. The one exception relates to the immunology programme; however, with final analyses, submission and publication, this project area will likely have met the original objectives, such that all major goals will have been met.

6.4 (a) Peer-reviewed journal publication(s) arising directly from this 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. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						

See Appendix 3, which contains a list of articles that resulted because of TRS support. Also see Appendix 4, where the actual acknowledgements or statements regarding why acknowledgements were not included are explained. In some cases, TRS support was not originally cited (publications from K. Boheler prior to 2014), because he was a U.S. government employee and could not accept TRS funds; however, some of the work could be attributed to TRS funds, as these were studies that took place in anticipation of his arrival in Hong Kong. Appendix 6 has pdf files (where available) of publications coming from this TRS funded project.

(b) Recognised international conference(s) in which paper(s) related to this project was/were delivered:
(Please attach a copy of each conference abstract)

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

Please see Appendix 3 for a list of conferences in which papers were delivered. Please note, a copy of conference abstracts prior to 2016 should already be on file with the RGC. Abstracts from 2016 are attached to Appendix 3.

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

While reviewing a number of publications that had been originally attributed to TRS funding (in prior reports), we discovered that some manuscripts did not acknowledge the RGC. It is unclear why this occurred, and this information has been indicated in Appendix 2 and Appendix 3. For other instances where TRS funding was not acknowledge, explanations are given.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

This project has cemented the collaboration of several investigators, first as part of this TRS funded project, second to complete on-going research, third cooperation in the development of new project areas, and conversations and applications for joint research funding.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation

See Appendix 3 for a list of trained research students

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

N/A

6.8 Other education activities and/or training programmes developed:

Most of the educational activities dealt with the training of students in stem cell biology. In part due to the infrastructure that was set up, a number of other researchers from multiple institutes have come to learn iPSC generation, CM differentiation, and other applications. This has involved researchers not only from Hong Kong, but also from China.

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC, which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

Due to perceived delays in the completion of the project and serious concerns regarding Projects 3 and 4, the RGC and M&A Panel requested that the Deputy PC monitor the activities of the PIs and co-Is closely during the 5th year. A preliminary set of Milestones was submitted to the RGC for review. These were approved, and updates to our final set of Milestones approved by the Management Committee can be found in Appendix 2 for review. Although the Deputy PC was in regular contact (phone, email, person) with all of the PIs/co-Is involved with designated milestones, a number of manuscripts listed in the Milestones have not been published. In some cases, this is due to revisions, with resubmission, but not yet manuscript acceptance. One key component that was delayed was associated with Project 3, Aim 1, particularly after C. Chan left the program of HKU and failed to complete the writing and submission of several manuscripts. The Deputy PC asked the PC to ensure completion, and he was told that publications would be forthcoming as set forth in the Milestones. Ultimately, some of the data were incorporated into other manuscripts; however, several of these planned papers do not seem to have ever been submitted. Due to M&A Penel concerns with the Immunology section, funds were made available to G. Chan (a PI) to develop and complete relevant Immunological experiments that were reviewed by the SAB member Prof. Colin Stewart. As this did not receive funding until after March of 2016, this project area saw significant delays; however, it is on course for completion. Ultimately, some of the transriptomic data were incorporated into 3 manuscripts for Project 1 during revision, and the data on autologous transplantation data added to the manuscript in Project 4, Aim 2; two separate manuscripts have also been prepared and submitted. Updates for the unpublished but nearly completed work with Godfrey Chan are provided in Appendices 1 and 2. Finally,

Project 4 has seen good progress; two manuscripts relevant to this project have been published. A third manuscript has also been submitted.

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

The project was managed from the outset by the PC rather than by the management team. At the recommendation of the RGC and the Monitoring and Assessment (M&A) Panel, major administrative changes were put into place during the 5th year. A Deputy PC was appointed, who handled all major aspects of this project since December 2015. As part of the Management Strategy, the Deputy PC visited the affiliated University campuses to speak with the TRS-associated PIs, co-Is, and students and to assess scientific progress. The Deputy PC has been in regular contact with the PIs and co-Is of this project, and he emails for written confirmation regarding progress on the Milestones. Following recommendations by Associate Dean, SY Leung and by the Scientific Advisory Board (SAB), the distribution of funds was modified. The most recent SAB review of the program was held on 19 May 2016 and positive comments were conveyed to the Deputy PC regarding the overall Positive Attitude and improved focus. Based on direct feedback from PIs, it appears that there has been significant improvement in the coordination, management, accountability, and financial monitoring of the project following the appointment of Prof. Kenneth Boheler as the Deputy PC.

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7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Yes. ITC funding was awarded for a cooperative project between NovoHeart and HKU, and researchers at HKUST have secured additional ITC funding for their projects. HMRF funds were awarded to PIs associated with this TRS funded project due to results and techniques that came from this project. Additional funding may be forthcoming after additional grants have been submitted.

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Yes, many of which are listed among the conferences 6.4 b.

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

A number of PIs involved with this program were already part of leadership positions and these have continued, in part due to the continued productivity made possible by TRS support.

7.4 Any documentary proof of the application of technologies arising directly from this project?

Documentary proof for the application of several technologies is mostly in the form of publications, particularly our methods manuscripts/chapters/papers which have subsequently been cited in the literature

7.5 Other awards and recognitions as a result of this project (please specify):

- Sarah Ho was awarded a prize for best poster at The 11th Symposium on Calcium Signaling in China (SCSC) held in Zunyi, China from 21-24 July 2016. The title of her poster was *"Expression and reconstitution of the bioluminescent Ca^{2+} reporter aequorin in human embryonic stem cells, and determination of the presence of functional IP_3 receptors at the earliest stages of their differentiation into cardiomyocytes."*
- Helen Hao won best poster at the 3rd Macau Symposium on Biomedical Sciences for her presentation entitled "*Store-operated Ca2+ entry (SOCE) via TRPC3 is required for cell proliferation, differentiation, and survival of undifferentiated Sox1-GFP 46C mouse embryonic stem cells (mESCs) as well as those undergoing neural differentiation"*.
- The cover of the Science China Life Sciences Journal (Chan H et al, 2016) utilized a figure generated as part of this project.

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

HKU - The formation of a spin-out company (NovoHeart), located at the Hong Kong Science & Technology Park (HKSTP) along with the first $21M R&D project approved by the Innovation Technology Fund (ITF), which will provide new jobs, including some for graduates of this TRS program. The research output will facilitate the development of commercial ventures and may include activities associated with drug screening for Pharma and other activities not covered by the present TRS grant. Through a novel drug repurposing approach based on CSC-Technology outputs, we identified a small molecule STF31 that is preferentially toxic to pluripotent stem cells either alone or as part of mixed culture. Based on these findings, we have expanded our analyses to other NAMPT inhibitors to test their effects on differentiated cell types implicated in regenerative medicine the results of which have led to inquiries from Pharma about possible applications. It has also led to several collaborative projects, including with external International organizations (Stem Cell Institute, Edinburgh). Other researchers in Hong Kong, the U.S.A. and in Europe have voiced an interest in collaborating with us due to our preeminent position in the field of cell surface capture technologies and application to stem cells.

HKUST-Hsing/Wu/Miller

The OECT platform is being considered for IP protection and possible commercial applications. Results from the 5th year will determine its ultimate utility. Platform development of the NanoLantern system in hPSCs may show that this system is optimal for long-term Ca2+ analyses

and if proven correct, the model systems will be invaluable for studying cardiomyocytes and other cells in vitro to gain better understanding of human developmental processes.

CityU-Sun/Cheng
The platforms and technologies developed will be widely available for continued use and development by other Universities. While the modified RNA studies are on-going, if it proves robust with limited cell-to-cell variability, then this technology will be suitable for single cell analysis and applications to human development and reprogramming.

CUHK-Yao
We utilized the human embryonic stem cell-derived cardiomyocytes to establish the human disease model of cardiac hypertrophy. We also used human embryonic stem cell-derived cardiomyocytes to investigate the mechanism of contraction-facilitated cardiomyocyte alignment during human heart development. These models for human heart diseases and/or heart development are of potential for development into future therapeutic usage.

8.2 Others (please specify):

Nil

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Yes, new ideas have developed. First, the results have been instrumental in supporting ITF supported research with NovoHeart. The bioengineered tissues will likely be of research value for years to come, both as part of this private enterprise and for use in basic research. Second, two new HMRF funded projects have come from the Cell surface capture analyses, as well as new collaborations within HK and in China. Third and while the initial experiments with apo-aequorin did not work, the development of the Nano-lantern system, first outside of this project and then through its application to this project as a potential innovation, will likely lead to new lines of investigation and future research projects. Fourth, we believe that the organic electrochemical transistor array we developed from this project may also be useful for the development of flexible brain probe for brain-machine-interface applications. Other ideas and innovations will likely develop from the results published as part of this project.

9.2 Whether there are new projects evolved directly from this project?

A number of projects have received ITF (ITS/131/13FX), HMRF (several grants) and other grant funding have developed directly from this project. These projects are either building upon results coming from this project or have taken advantage of the methodological breakthroughs that resulted from this project. At HKUST, we have a newly funded ITF-tier 3 project of 1.3 Million entitled “Macroporous Organic Electrochemical Transistor Mesh as a Flexible Brain Probe”, which is expected to start in Jan. 2018. Other applications for funding have been or are planned for submission.

9.3 Whether there are new collaborations developed directly from this project?

Yes. Some of the developing collaborations involve investigators who were actively involved with this project and who will continue their research initiatives. Other collaborations (national and international) have developed due to results and research advances made because of funding of this project.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

The total amount of money is currently unknown, as not all grant funding levels from the various researchers is available. But funds have been obtained from the ITC, HMRF and sources in main land China. These sum to an amount of >HK$22 million. Other private or commercial funds may become available, as negotiations are still under way.

Theme-based Research Scheme
Cell-Based Heart Regeneration-Layman's Summary

Heart diseases are a major cause of death worldwide. Loss of cardiomyocytes (CMs) due to aging or diseases is irreversible. Current therapeutic regimes are palliative; in end-stage heart failure, transplantation remains the last resort but is significantly hampered by a severe shortage of donors. Human embryonic stem cells (hESCs) can self-renew while maintaining their pluripotency to differentiate into all cell types, including CMs. Direct reprogramming of adult somatic cells to induced pluripotent stem cells (iPSCs) has been achieved. The availability of hESC/iPSCs has enabled researchers to gain novel biological insights and to pursue heart regeneration. Despite these promises, substantial hurdles remain for translating into cell-based therapies and other applications (e.g., disease modeling, cardiotoxicity and drug screening). Based on our team's own work in the past decade, we have identified major gaps: hESC-CMs have immature properties, small physical size, absence of ordered organization, poorly-defined immunobiology and sub-lineage specification, uncertain safety and efficacy. To address these, we have assembled a multi-disciplinary team of world-class experts to work collaboratively on a 5-year TRS project entitled 'Cell Based Heart Regeneration'

Research highlights and Impacts
Some of the major objectives achieved in this programme include advances in *Engineering of Human Cardiac Muscle and Chamber* including the development of Engineered Cardiac Tissues (ECTs) using technologies shared between Hong Kong and the U.S.A. (Costa, Hajjar, Li), and new technologies/platforms or technological advancements developed in Hong Kong (BP Chan; I-M Hsing; J Qu; A Miller). Extensive basic science analyses were performed examining the mechanical and electrophysiological traits of cells and cell constructs. The *Biology of Cell Engineering* project led to the development of novel techniques for Ca^{2+} imaging. Basic science analyses of TRPV channels has led to a greater understanding and appreciation of their diverse roles in biology and particularly with emphasis on hPSC-CMs. This area of research has been accumulating citations and gainin international recognition. This project has also led to the development of organic electrochemical transistors (OECTs) and use of microfluidic technologies for single cell manipulation (patents pending).
A spin-off company (NovoHeart), located at the Hong Kong Science & Technology Park (HKSTP) along with the first R&D project approved by the Innovation Technology Fund (ITF) ($10M with $11.2M matching fund from NovoHeart) was established that has provided jobs for some graduates of this TRS program and others in Hong Kong. These scientific advances and interactions with companies will facilitate further basic research endeavors and the development of commercial ventures. Specifically, new collaborations and activities involving the development of drug screening for Pharma not covered by the present TRS grant have been initiated.

Other major achievements
Publications and awards: A total of 115 peer-reviewed articles, chapters or other papers and 34 international conference papers have been published. Funding of two ITF Projects, ($10M and $5.661M) on two new projects using the various cardiac tissue constructs developed in this TRS project.
Education and training: During the 5 year project period a total of 6 MPhil and 21 PhD students along with 11 Postdoctoral fellows have been trained. Seven of the trainees have been promoted to faculty in local and overseas academic institutions. Nine trainees have pursued a career in industry after their training.

The above summary is written mainly by the project team. The views expressed in the summary do not necessarily represent those of the University Grants Committee/Research Grants Council.