RGC Ref.: N_HKU725/16 NSFC Ref.: 51661165014 (please insert ref. above)

The Research Grants Council of Hong Kong NSFC/RGC Joint Research Scheme <u>Joint Completion Report</u>

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Study of the degradation behavior and osteogenic effects of Mg-Si based biodegradable materials

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

	Hong Kong Team	Mainland Team
Name of Principal	Prof. Kenneth M. C. Cheung	Prof. Yufeng Zheng
Investigator (with title)		
Post	Head & Clinical Prof	Professor
Unit / Department /	Dept. of Ortho and Trauma	Dept. of Materials Sci and
Institution	HKU	Eng PKU
Contact Information	cheungmc@hku.hk	yfzheng@pku.edu.cn
Co-investigator(s)	Prof. Kelvin W.K. Yeung, Dr.	Dr. Yan Cheng, Dr. Huafang
(with title and	Karen H.M. Wong, HKU	Li, Ms. Wenting Li, PKU
institution)		, ,

3. Project Duration

	Original	Revised	Date of RGC/
			Institution Approval (must be quoted)
Project Start date	1-1-2017	N/A	(musi de quoica)
Project Completion date	31-12-2020	N/A	
Duration (in month)	48	N/A	
Deadline for Submission of Completion Report		N/A	

Part B: The Completion Report

5. Project Objectives

- 5.1 Objectives as per original application
- 1. To fabricate and characterize the material and mechanical properties of magnesium-silicon (Mg-Si) based biomaterials e.g. micro-structure, hardness, corrosion behaviors and degradation rate etc. (by mainland team);
- 2. To systematically evaluate the toxicity, bioactivity and bio-functional properties e.g. osteoconductivity and osteoinductivity of the newly fabricated Mg-Si based biomaterials by using *in vitro* approaches (by HK team);

- 3. To comprehensively investigate the *in vivo* biological response (i.e. biomaterial degradation under *in vivo* condition, the ability of new bone formation under the influence of degradation products, tissue inflammatory etc.) of the new biomaterials by using established animal models (by HK Team).
- 5.2 Revised Objectives Not applicable

6. Research Outcome

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

The biomedical research reveals that the essential elements of human body, calcium (Ca), magnesium (Mg), silicon (Si), zinc (Zn) and strontium (Sr) play important roles during the mineralization, growth and vascularization of bone tissues. In the previous study on magnesium alloys, metallic elements Ca, Zn and Sr had been widely used as alloying elements for Mg, whereas non-metallic element, e.g., Si, was not the choice basically. Indeed, the osteogenic tendency of Si has been proven by a number of studies. Hence, our study aims to develop a series of biodegradable Mg-Si based biomaterials such as Mg-Si, Mg-Si-Ca, Mg-Si-Sr, Mg-Si-Zn, Mg-Si-Ca-Sr, Mg-Si-Ca-Zn, Mg-Si-Sr-Zn for bone surgery. In this study, we had investigated their microstructures, mechanical properties, in vitro and in vivo biodegradation behavior, biocompatibility and the ability to induce local bone formation. We discovered that the addition of Si in 0.2wt% and Ca in 1.0wt% in Mg matrix could significantly increase the mechanical strength and bio-adaptability of Mg-Si alloy. The addition of these two elements (Ca and Si) would accelerate the formation of a self-assembled, multilayered implant-tissue interface, which coordinated its biodegradation with the bone healing process. At the initial stage of implantation, a burst release of Mg²⁺ from Mg-Si-Ca alloy activated the monocyte-macrophage lineage, leading to an immune microenvironment favoring the recruitment of mesenchymal stem cells (MSCs) and initiation of osteogenic differentiation. With the formation of the biomimicking calcified matrix at the degrading bone-implant interface, the ion release kinetics of the Mg-Si-Ca alloy was turned down, leading to a new peri-implant microenvironment for osseointegration and osteogenesis by targeting the integrin signaling pathways in MSCs.

For the other Mg-Si based ternary alloys, Mg-0.2Si-1.0Sr could also significantly promote bone repair after implantation. Particularly, when its degradation completed, the cancellous bone adjacent to the implant in terms of bone mass and bone density had dramatically increased. We also observed that the degradation products from Mg-0.2Si-2.0Zn alloy might help thicken the cortical bone. We believed that the osteogenic mechanism could attribute to the release of magnesium ions, zinc ions and silicon ions in bone tissue microenvironment. The released cations altered the local bone

tissue microenvironment that might stimulate the NF-κB signaling pathway of macrophages. The activated macrophages then released a various of inflammatory factors, especially IL-1ra, CCL5 and IL-8, that could promote the proliferation and differentiation of osteoblasts. At the same time, the combined cations including Mg²⁺, Si²⁺, Zn²⁺ would significantly inhibit the expression of pro-inflammatory cytokines (M-CSF, IL-1β and IL-6) that might reduce osteoclast differentiation. For the Mg-Si quaternary alloys, we also identified that Mg-0.2Si-0.5Ca-0.5Sr, Mg-0.2Si-1.0Ca-2.0Zn and Mg-0.2Si-1.0Sr-2.0Zn alloys presented comparable biocompatibility *in vitro* and *in vivo*. However, they were not as good as other Mg-Si based ternary alloys.

Further details of these experimental data can be found in the publication list in Part C.

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

With the current research outcomes, we are still unable to address the long-term safety issue of these new biogradable metals that may require by the medical device registration authority such as China FDA. Hence, the next stage of work will be studying the long-term in vivo study by using large animal models in order to fulfill the requirements of product registration. Once we finish all the *in vivo* studies, we plan to submit another proposal with potential industrial partners in order to carry out human clinical trial.

7. The Layman's Summary

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

Patients with bone fracture or deformity are commonly treated by surgical intervention and fixation implants made of titanium alloys and 316L medical grade stainless steel. These implants are no longer useful to the patient following bone healing. If left inside the human body, the implant can potentially increase the stress-shielding effect at the bone-implant interface, eventually resulting in bone loss at the junction and failure of fixation. For this reason, the development of a biodegradable metal, e.g, magnesium (Mg), for bone fracture fixation is a desirable advance. Our works have demonstrated that some trace elements (e.g. calcium (Ca), zinc (Zn) and strontium (Sr), as well as silicon (Si) incorporated into Mg substrate) can enhance implant mechanical properties, while the these bioactive elements are able to induce the osteogenic differentiation and mineralization of bone cells, and to the growth and vascularization of bony tissue. The promising outcomes may help revolutionize the future development of orthopaedic implants, benefiting patients worldwide and eliminating the risks of a metallic implant left inside the body or the need to have further surgery to remove it.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project (Please attach a copy of each 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.)

Th	e Latest Status	of Publica	tions	Author(s)	Title and	Submitted to	Attached	Acknowledge	Accessible
Year of publication	Year of Acceptance	Under Review	Under Preparation	(bold the authors	Journal/ Book	RGC (indicate the	to this report (Yes	d the support of this Joint	from the institutional
2	(For paper accepted but not yet published)		(optional)	belonging to the project teams and denote the corresponding author with an asterisk*)	publishing details specified)	year ending of the relevant progress report)		Research Scheme (Yes or No)	repository (Yes or No)
2017				Wenhao WANG, Kelvin W.K. YEUNG*	Bone Grafts and Biomateri als Substitute s for Bone Defect Repair: A review. Bioactive Materials, 2(2017):	Yes, 2019	No	Yes	
2018				Paul K. Chu, Liming Bian, Shuilin Wu, Yufeng Zheng, Kenneth M. C. Cheung, Frankie Leung, Kelvin W.K. Yeung*	Precisely controlled delivery of magnesiu m ions thru sponge-lik e monodisp erse PLGA/na no-MgOalgin ate core-shell microsphe	Yes, 2019	No	Yes	

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2018	Wei Liu, Jinhua Li, Mengqi Cheng, Qiaojie Wang, Kelvin W. K. Yeung, Paul K. Chu, Xianlong Zhang	Zinc- Modified Sulfonate d Polyether etherketon e Surface with Immunom odulatory Function for Guiding Cell Fate and Bone Regenerat ion, Advanced Science, 2018, 5(10), 1800749		Yes	Yes	
2019	Ying Zhao, Kelvin W.K. Yeung	A	Not yet	Yes	Yes	

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9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered (Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/	Title	Conference Name	Submitted	Attached	A also avulados d	Associate
Place	1100	Conference ivalle	to RGC	to this	Acknowledged the support of	from the
2000 O. B			(indicate the		this Joint	institutional
		v	year ending	(Yes or No)		repository
			of the		Scheme	(Yes or No)
			relevant progress		(Yes or No)	
			report)			
	The role of	Australia-China	Yes, 2019	No	Yes	
November	magnesium	Conference of				
, 2018,	ion on	Tissue Engineering				
Cairns,	osteogenesis	and Regenerative				
Australia	and its	Medicine	,			
	clinical	(ACCTERM 2018)				
(applications					f
March,	Precise	ORS 2018 Annual	Yes, 2019	No	Yes	
2018, New	Control	Meeting				
Orleans,	Delivery of					
Louisiana,	Magnesium					
USA	Ions thru					
	Sponge-like					
	Monodispers					
	ed					
	PLGA/nano-					
	MgO-alginate					
	Core-shell					
	Microsphere	*				
	Device to					
	Enable In-situ					
	Bone					
	Regeneration					

10. 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
Zhengjie, Lin	PhD	September 1, 2014	Aug 31, 2018

11. Other impact (e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)

Award

 COA Orthopaedic Young Researcher Award 2nd Prize selected by The 13th Annual Congress of Chinese Orthopaedic Association, Xiamen, PR of China, 21st-24th Nov, 2018.

Patent

- (1) 中国专利, 郑玉峰(#); 杨宏韬; 张泽川; 沈丹妮, 一种Zn-Li-Mg系锌合金及其制备方法与应用, 申请, 2018.3.23, CN201810244009
- (2) 中国专利, 郑玉峰(#); 杨宏韬; 张泽川, 一种Zn-Li-Mn系锌合金及其制备方法与应用, 申请, 2018.3.23, CN201810243999
- (3) 中国专利, 郑玉峰(#); 李文婷(#); 成艳(#); 夏丹丹; 刘晓, Mg-Si-Ca-Zn系镁合金及其制备方法与应用,申请, 2019.4.18, CN201910312727.6
- (4) 中国专利, 郑玉峰(#); 李文婷(#); 成艳(#); 夏丹丹; 刘晓, 一种含Sr的镁合金, 申请, 2019.4.18, CN201910312395.1
- (5) 中国专利, 郑玉峰(#); 边东; 刘嘉宁; 成艳(#); 夏丹丹, 一种含Mg-RE系镁合金及其制备方法与应用, 申请, 2019.2.19, CN201910121354.4
- (6) 中国专利, 鄢江龙; 夏丹丹; 郑玉峰(#); 成艳(#), 一种骨科植入器械表面涂层的制备方法, 申请, 2020.8.14, CN202010582000.2

Scholarly books, monographs and chapters

- Zheng yufeng(#); Xu Xiaoxue; Xu Zhigang; Wang Junqiang; Cai Hong; Metallic Biomaterials: New Directions and Technologies, Wiley-VCH Verlag GmbH & Co. KGaA, 2017.
- **12. Statistics on Research Outputs** (Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)

	Peer-reviewed	Conference	Scholarly books,	Patents awarded	Other research
	journal	papers	monographs and		outputs
	publications		chapters		(Please specify)
No. of outputs	13	2	1	0	COA
arising directly					Orthopaedic
from this research					Young
project [or					Researcher
conference]					Award