Project number: T13-402/17-N

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *Nov 2017*
Project completion date: *June 2023*

1. Project Title: Functional Bone Regeneration in Challenging Bone Disorders and Defects

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)	QIN, Ling / Choh Ming Li Professor[1]	Dept. of Orthopaedics and Traumatology, The Chinese University of Hong Kong (CUHK)	16
Co-Principal Investigator(s)	Cheung, Wing Hoi / Professor[2]	Dept. of Orthopaedics and Traumatology, CUHK	12
	Li, Gang / Professor	Dept. of Orthopaedics and Traumatology, CUHK	12
	Lu, Jian / Chair Professor of Mechanical Engineering	Dept. of Mechanical and Biomedical Engineering Director of Centre for Advanced Structural Materials, City University of HK	6
	Ruan, Ye Chun[3] / Asso. Professor[4]	Interdisciplinary Division of Biomedical Engineering, PolyU	18
	Yung, Patrick Shu Hang / Clinical Professor	Dept. of Orthopaedics and Traumatology, CUHK	6
Co-Investigator(s)	Bian, Liming[5] / Asst.	Dept. of Mechanical and	12

	Professor	Automation Engineering, CUHK	
	Feng, Bo / Asst. Professor	School of Biomedical Sciences, CUHK	12
	Guo, Xia[6] / Asso. Professor	Dept. of Rehabilitation Sciences, The Hong Kong Polytechnic University (PolyU)	0
	Lu, William Wei Jia / Professor	Department of Orthopaedics & Traumatology, HKU	6
	Tang, Ning / Clinical Consultant	Dept. of Orthopaedics and Traumatology, PWH	6
	Zheng, Yong Ping / Professor	Interdisciplinary Division of Biomedical Engineering, PolyU	8
Collaborators	Alini, Mauro / Professor	Head of Musculoskeletal Regeneration, AO Foundation	N.A.
	Cao, Xu / Professor	Dept. of Orthopaedics Surgery, Johns Hopkins School of Medicine	N.A.
	Ding, Ming / Professor	Dept. of Orthopaedic Surgery and Traumatology, Odense University Hospital, Institute of Clinical Research, University of Southern Denmark	N.A.
	Witte, Frank / Professor	Julius Wolff Institute and Center for Musculoskeletal Surgery; Charité - Universitätsmedizin Berlin, Germany	N.A.

 # 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.

1. Meanwhile, Professor Qin Ling was honoured with Choh- Ming Li Professorship in May 2022.

2. Meanwhile, Professor Cheung Wing Hoi was promoted to Full Professor in Aug 2021.

3. Meanwhile, the change of Co-PI of Prof. Hsiao Chang Chan to Prof. Ye Chun Ruan was approved on Oct 18, 2017.

4. Meanwhile, Prof. Ye Chun Ruan was promoted to Associate Professor in July 2022.

5. Meanwhile, Prof. Liming Bian resigned from CUHK and employed at the School of Biomedical Sciences and Engineering, South China University (Effective on June 15, 2021).

6. Meanwhile, Prof. Xia Guo retired from PolyU and was released from the current project. This was approved on May 7, 2019.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. BMSC expansion and modulations	100%	Delay from 1 Nov 2022 to 30 June 2023 was justified and approved in our previous interim reports that is due to COVID 19 that prevent our implementation of large animal experiments
2. In vitro experiments	100%	
3. Disease-specific biomaterials fabrication	100%	

Objectives*	Percentage achieved	Remarks**
4. Biosafety tests by regulatory body certified testing center(s)	100%	
5. *In vivo* experiment in small animal models	100%	
6. *In vivo* experiment in large animal models	100%	

* *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

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.))

Per our study objectives or milestones, here we listed up 7 very exciting accomplishments cited from over 100 publications generated from the current TRS with innovative elements, scientific contribution and translational potential for clinical applications and commercialization.

6.1.1 Mg facilitates the healing of bisphosphonate-related atypical femoral fractures

Bisphosphonates (BPs)-associated atypical femoral fractures (AFFs) present with impaired fracture healing, yet the underlying mechanism is unclear, which prevents the development of effective therapy. Peripheral sensory nerve has been shown to regulate fracture healing via releasing neuropeptides. Here we show that long-term BPs pre-treatment leads to fracture non-union in rats, characterized by reduced expression of calcitonin gene-related peptide (CGRP, a predominant type of neuropeptides) and abundant fibrous tissues in the non-bridged fracture gap, mimicking clinical AFFs. By using single-cell RNA-sequencing, long-term BPs treatment was identified to promote transition of progenitor cells into a specific cluster of fibroblasts that actively deposit dense extracellular matrix (ECM) to prevent fracture callus bridging. Administration of exogenous CGRP at early stages of fracture repair, in contrast, eliminates the ECM-secreting fibroblast cluster, attenuates fibrogenesis, and facilitates callus bridging, suggesting CGRP is a promising agent to facilitate AFF healing. Accordingly, we have developed an innovative magnesium (Mg) containing hybrid intramedullary nail fixation system (Mg-IMN) to effectively rescue BPs-impaired fracture healing via elevating CGRP synthesis and release. Such device optimizes the fracture healing in BPs-pretreated rats, comparable to direct administration of CGRP. These findings address the indispensable role of CGRP in advancing the healing of AFFs and develop translational strategies to accelerate AFF healing by taking advantage of the CGRP-stimulating effect of Mg-based biodegradable orthopaedic implant. The study also indicates fibrosis could be targeted by augmenting CGRP expression to accelerate fracture healing even under challenging scenarios where fibroblasts are aberrantly activated.

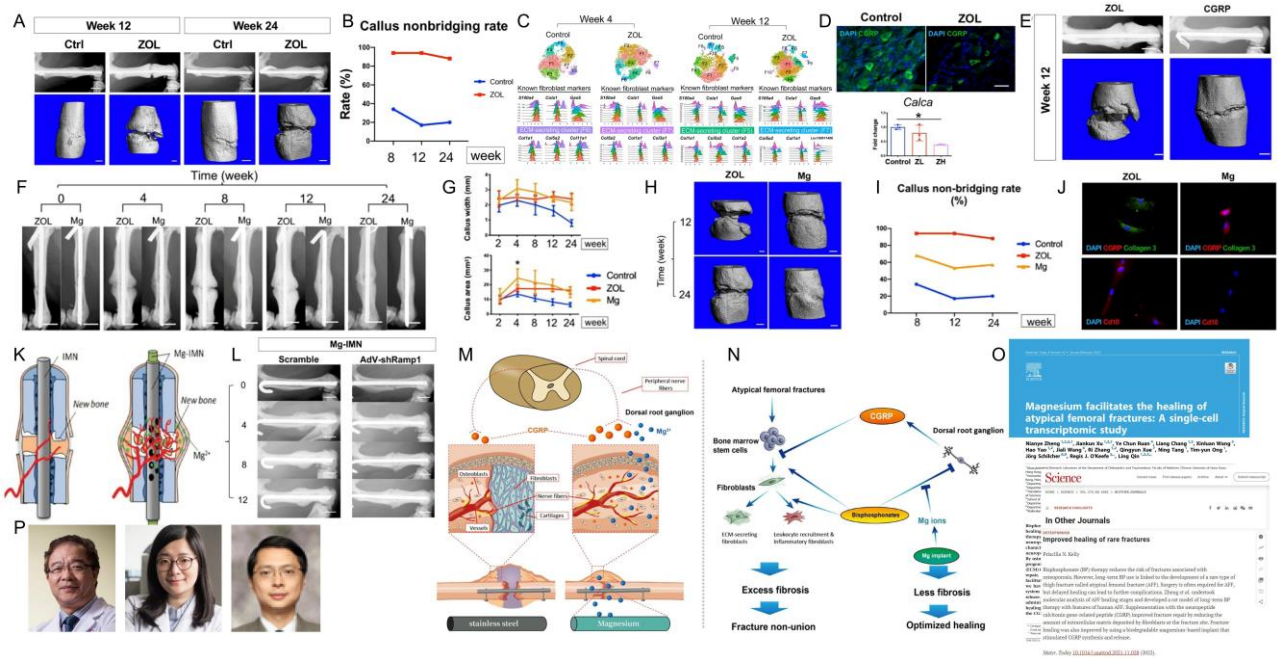

Figure 1. Mg facilitates the healing of bisphosphonate-related atypical femoral fractures (Selected from Zheng NY, et al., ***Materials Today,*** Vol 52, 2022). **(A)** Zoledronate (ZOL) treatment induced non-bridging of fracture gap as shown by X-ray and micro computed tomography. **(B)** The

non-healing rate of ZOL-treated rats at week 12 and 24 was 94% and 88%, respectively. **(C)** Upper panel: scRNA-seq revealed heterogenous cell clusters on t-distributed stochastic neighbor embedding (t-SNE) plot. Middle panel: all the populations were fibroblasts based on expression of known fibroblast markers. Lower panel: a unique cluster existed across all groups, the gene markers of which were associated with extracellular matrix (ECM) production. The top 3 expression markers for each group were plotted. **(D)** Upper panel: Immunofluorescent images of CGRP expression in DRG at 4 wpf. Scale bar, 50 μm. Lower panel: RT-PCR results for CGRP expression in primary DRGs *in vitro* treated with either low (500 nM, ZL) or high concentration (5 μM, ZH) of ZOL for 2 days. **(E)** X-rays and 3D reconstruction of CT data. **(F)** Representative X-rays of ZOL-pretreated rats fixed with Mg-IMN (Mg group). **(G)** X-ray-based calculation of callus area and callus width, n = 4–6. **(H)** Representative μCT reconstruction of Mg group rats. Scale bar, 1 mm. **(I)** Radiograph-based calculation of callus non-bridging rate. **(J)** Fibrogenic induction of BMSCs isolated from Mg group rats at 4 wpf. Left panel: Immunofluorescent staining of collagen 3, Cd10, and CGRP on day 10 of fibrogenic induction. Scale bar, 50 μm. **(K)** Schematic diagram of the Mg-IMN. The pure Mg pin inside the stainless-steel IMN (SS-IMN) gradually released Mg ions through the vents into surrounding bone tissues to stimulate the release of CGRP, which in turn promoted bone formation and angiogenesis while prevented fibrosis. **(L)** Radiograph showed that *Ramp1* shRNA-adenovirus treated rats (AdV-shRamp1) displayed non-bridged fracture gaps at 12 wpf, opposing to the scramble group where callus bridged smoothly. **(M)** Mg ions released from the magnesium containing intramedullary nail (Mg-IMN) stimulates CGRP release from the dorsal root ganglion into the fracture site, resulting in optimized neovascularization, lesser fibrosis, and bridging of the fracture callus. **(N)** At cellular level, long-term BPs treatment diverts bone marrow stem cells (BMSCs) into a fibroblastic cell fate by a direct effect of BPs as well as BPs-induced reduction in CGRP release from the dorsal root ganglion. This leads to an increase in number of pathogenic fibroblasts that actively secret extracellular matrix and/or mediate inflammation, contributing to excess fibrosis and non-union of AFFs. Mg-IMN, in contrast, promotes AFF healing by stimulating CGRP release. **(O)** The published page of this study, that was highlighted in ***Science*** (https://www.science.org/doi/10.1126/science.ada1187). **(P)** The authors from this TRS team for this study **(Prof. Ling QIN, Prof. Yechun RUAN and Dr. Ning TANG).**

The present study, by establishing a rat model of delayed fracture healing that phenocopied clinical AFF specimens, reveals that BPs treatment suppresses CGRP expression and impairs fracture union by generating aberrant fibrous tissues at the fracture gap. To the best of our knowledge, it is the first time scRNA-seq was applied to identify that ZOL can shunt the fate of myeloid progenitor cells toward fibroblasts that actively secreted extracellular matrix and subsequently impeded fracture union. Conversely, replenishing exogenous or endogenous CGRP by surgical fixation with Mg-IMN attenuated the unfavourable effect of ZOL on fibroblast population, resulting in prominently decreased fracture non-union rate. A translational approach to enhance AFF healing by using Mg-IMN has been validated with appreciation of the previously un-recognized anti-fibrotic effect of CGRP. More importantly, eliminating fibroblasts by magnesium-based biometal could be a novel therapeutic approach to accelerate fracture healing/bone regeneration where over-activated fibroblasts are involved (Zheng NY, et al., ***Materials Today,*** Vol 52, 2022, https://doi.org/10.1016/j.mattod.2021.11.028).

6.1.2 Mg-encapsulated injectable hydrogel and 3D-engineered polycaprolactone conduit facilitate peripheral nerve regeneration

Peripheral nerve injury is a challenging orthopaedic condition that can be treated by autograft transplantation, a gold standard treatment in the current clinical setting. Nevertheless, limited availability of autografts and potential morbidities in donors hampers its widespread application. Bioactive scaffold-based tissue engineering is a promising strategy to promote nerve regeneration. Additionally, magnesium (Mg) ions enhance nerve regeneration; however, an effectively controlled delivery vehicle is necessary to optimize their in vivo therapeutic effects. Herein, a bisphosphonate-based injectable hydrogel exhibiting sustained Mg^{2+} delivery for peripheral nerve

regeneration is developed. It is observed that Mg^{2+} promoted neurite outgrowth in a concentration-dependent manner by activating the PI3K/Akt signalling pathway and Sema5b. Moreover, implantation of polycaprolactone (PCL) conduits filled with Mg^{2+} -releasing hydrogel in 10 mm nerve defects in rats significantly enhanced axon regeneration and remyelination at 12 weeks post-operation compared to the controls (blank conduits or conduits filled with Mg^{2+}-absent hydrogel). Functional recovery analysis reveals enhanced reinnervation in the animals treated with the Mg^{2+}-releasing hydrogel compared to that in the control groups. In summary, the Mg^{2+}-releasing hydrogel combined with the 3D-engineered PCL conduit promotes peripheral nerve regeneration and functional recovery. Thus, a new strategy for challenging peripheral nerve injuries is proposed.

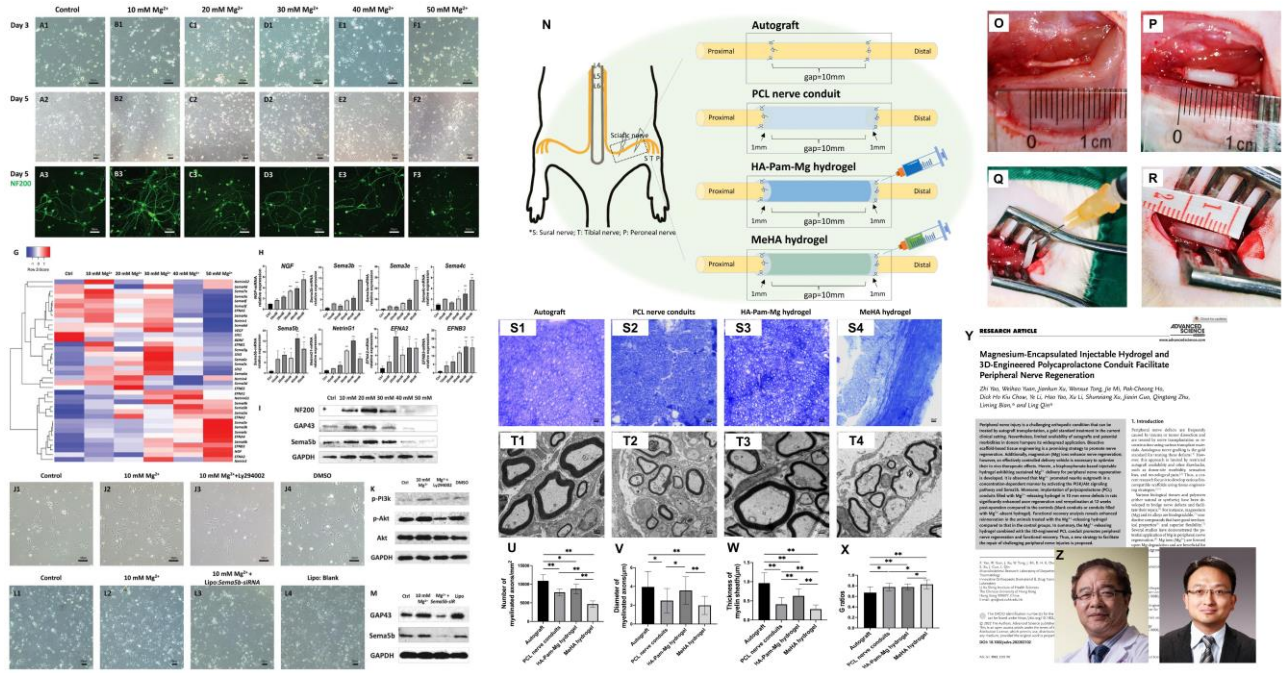

Figure 2. Mg-Encapsulated Injectable Hydrogel and 3D-Engineered Polycaprolactone Conduit Facilitate Peripheral Nerve Regeneration (Selected from Yao Z, et al., ***Advanced Science,*** June 2022). **(A–F)** Primary culture of DRG neurons treated with varying Mg^{2+} concentrations. **(G)** Gene expression heatmap of axon guidance molecules and neurotrophic factors. **(H)** mRNA expression levels. **(I)** Protein expression levels of NF200, GAP43, Sema5b, and GAPDH assessed by western blotting. **(J)** Primary culture of DRG neurons treated with additional Mg^{2+} ions, LY294002, or DMSO. **(K)** Protein expression levels in four groups. **(L)** Primary culture of DRG neurons with additional Mg^{2+}, Lipo: *Sema5b*-siRNA or Lipo: Blank; **(M)** Western blot results of GAP43, Sema5b expression in four groups. DRG, dorsal root ganglia. **(N)** Surgical procedures performed on SD rats belonging to four groups, that is, the autograft, PCL nerve conduit, HA-Pam-Mg hydrogel, and MeHA hydrogel groups. **(O)** Repair of a nerve defect using autografts. **(P)** Repair of a nerve defect using PCL nerve conduits. **(Q, R)** Surgical procedures in the HA-Pam-Mg hydrogel and MeHA hydrogel groups, wherein first one nerve stump is sutured, followed by injection of the hydrogels into the nerve conduit cavities. HA, hyaluronic acid; Pam, pamidronate; Mg, magnesium; SEM, scanning electron microscopy; MeHA, methacrylated HA. **(S)** Toluidine blue staining of transverse sections of nerve grafts harvested 12 weeks post-surgery. **(T)** TEM analysis of the remyelinated axons. **(U)** Number of the myelinated axons. **(V)** Diameter of the myelinated axons (μm). **(W)** Thickness of the new myelin sheath (μm). **(X)** G-ratio. Data are expressed as the mean ± standard deviation. One-way ANOVA followed by Tukey's *post-hoc* test is performed to analyze statistical significance. **(Y)** The cover page of this published study. **(Z)** The authors from this TRS team for this study **(Prof. Ling QIN and Prof. Liming BIAN).**

In this study, we demonstrated the role of Mg^{2+} in promoting axonal outgrowth by activating Sema5b and the PI3K/Akt pathway, and Mg^{2+} promoted neurite outgrowth in a concentration - dependent manner. Thus, our innovative HA-Pam-Mg hydrogel serves as a scaffold that physically supports axonal outgrowth and induces local Mg^{2+} release. Consequently, it accelerates early nerve

regeneration leading to better functional recovery from peripheral nerve injury in a rat model (Yao Z, et al., ***Advanced Science,*** June 2022, https://doi.org/10.1002/advs.202202102).

6.1.3 Combination of magnesium ions and vitamin C alleviates synovitis and osteophyte formation in osteoarthritis of mice.

We previously demonstrated that magnesium ions (Mg^{2+}) were a novel therapeutic alternative for osteoarthritis (OA) through promoting the hypoxia inducible factor-1α (HIF-1α)-mediated cartilage matrix synthesis. However, oxidative stress can inhibit the expression of HIF-1α, amplify the inflammation that potentially impairs the therapeutic efficacy of Mg^{2+} in OA. Vitamin (VC), a potent antioxidant, may enhance the efficacy of Mg^{2+} in OA treatment. This study aims to investigate the efficacy of combination of Mg^{2+} and VC on alleviating joint destruction and pain in OA. Anterior cruciate ligament transection with partial medial meniscectomy induced mice OA model were randomly received intra-articular injection of either saline, $MgCl_2$ (0.5 mol/L), VC (3 mg/ml) or $MgCl_2$ (0.5 mol/L) plus VC (3 mg/ml) at week 2 post-operation, twice weekly, for 2 weeks. Joint pain and pathological changes were assessed by gait analysis, histology, western blotting and micro-CT. Mg^{2+} and VC showed additive effects to significantly alleviate the joint destruction and pain. The efficacy of this combined therapy could sustain for 3 months after the last injection. We demonstrated that VC enhanced the promotive effect of Mg^{2+} on HIF-1α expression in cartilage. Additionally, combination of Mg^{2+} and VC markedly promoted the M2 polarization of macrophages in synovium. Furthermore, combination of Mg^{2+} and VC inhibited osteophyte formation and expressions of pain-related neuropeptides. Intra-articular administration of Mg^{2+} and VC additively alleviates joint destruction and pain in OA. Our current formulation may be a cost-effective alternative treatment for OA.

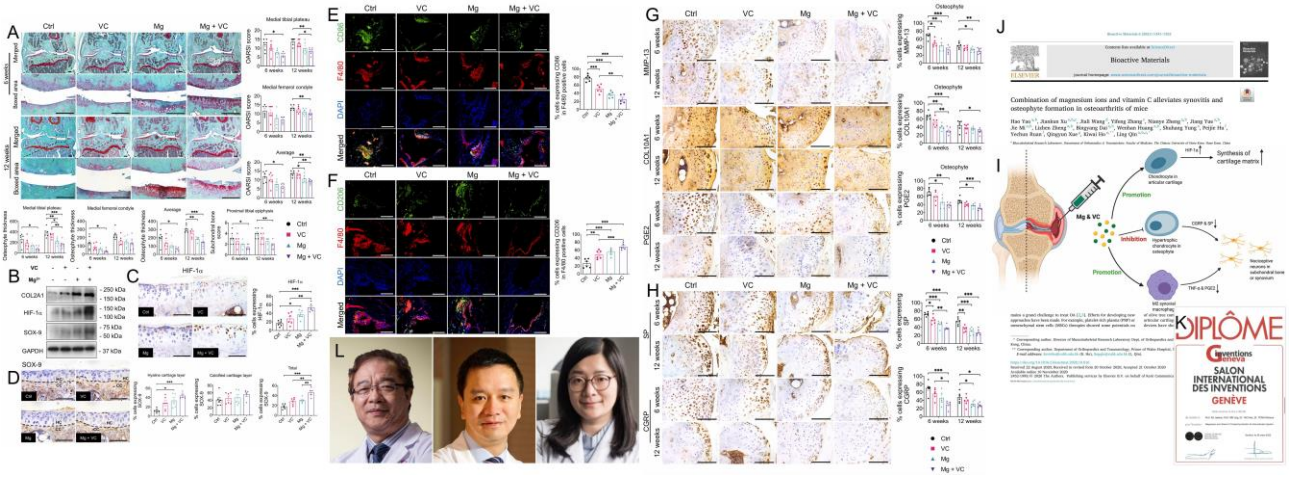

Figure 3. Combination of magnesium ions and vitamin C alleviates synovitis and osteophyte formation in osteoarthritis of mice (Selected from Yao H, et al., ***Bioactive Materials,*** 2020 Nov 10;6(5):1341-1352). **(A)** Left, Safranin O/Fast green staining showed the changes on cartilage in each group at week 6 and 12 post-treatment. Scale bar, 250 μm. Right and bottom, cartilage degeneration, subchondral bone changes and osteophyte development were quantified by OARSI scoring system. **(B)** Western Blotting showed the expressions of COL2A1, HIF-1α and SOX-9 in dissected cartilage tissue at week 1 post-treatment. **(C)** Left, IHC staining showed the expressions of HIF-1α in articular cartilage at week 6 post-treatment. Scale bar, 100 μm. Right, quantification of HIF-1α positive cells in cartilage at week 6 post-treatment. **(D)** Left, IHC staining showed the expression of SOX-9 in hyaline cartilage (HC) and calcified cartilage (CC) layer at week 6 post-treatment. Right, quantification of SOX-9 positive cells in either HC or CC layer. Dash line, tide mark. **(E)** Left, IF staining showed the expressions of CD86 (M1 macrophage marker) and F4/80 (macrophage marker) in synovium at week 6 post-treatment. Scale bar, 100 μm. Right, quantification of CD86 in F4/80 positive cells in synovium at week 6 post-treatment. **(F)** Lift, IF staining showed the expressions of CD206 (M2 macrophage marker) and F4/80 in synovium at week 6 post-treatment. Scale bar, 100 μm. Right, quantification of CD206 in F4/80 positive cells in

synovium at week 6 post-treatment. **(G)** Left, IHC staining showed the expressions of MMP-13, COL10A1 and PGE2 in osteophyte at week 6 and 12 post-treatment. Scale bar, 100 μm. Right, quantification of MMP-13, COL10A1 and PGE2 positive cells in osteophyte at the indicated time points. **(H)** Left, IHC staining showed the expressions of SP and CGRP in osteophyte at week 6 and 12 post-treatment. Scale bar, 100 μm. Right, quantification of SP and CGRP positive cells in osteophyte at the indicated time points. **(I)** Mg^{2+} and VC showed additive effects to significantly alleviate the joint destruction and pain. The efficacy of this combined therapy could sustain for 3 months after the last injection. We demonstrated that VC enhanced the promotive effect of Mg^{2+} on HIF-1α expression in cartilage. Additionally, combination of Mg^{2+} and VC markedly promoted the M2 polarization of macrophages in synovium. Furthermore, combination of Mg^{2+} and VC inhibited osteophyte formation and expressions of pain-related neuropeptides. **(J)** The cover page of this published study. **(K)** The Geneva Inventions Award of this study. **(L)** The authors of TRS team for this study **(Prof. Ling QIN, Prof. Patrick S.H. YUNG, and Prof. Yechun RUAN).**

In this study, we demonstrated that intra-articular injection of Mg^{2+} and VC could efficaciously alleviate the joint pathology and pain-related behaviours in ACLT+PMM induced mice OA model. The efficacy of such combination therapy could sustain for 3 months after the last treatment. We demonstrated that VC significantly enhanced the promotive effect of Mg^{2+} on the expression of HIF-1α in cartilage. In addition, intra-articular injection of Mg^{2+} and VC markedly promoted the M2 polarization of synovial macrophages. Moreover, combination of Mg^{2+} and VC also inhibited the osteophyte formation and the productions of pain-related neuropeptides (SP and CGRP). (Yao H, et al., Bioactive Materials, 2020 Nov, https://doi.org/10.1016/j.bioactmat.2020.10.016)

6.1.4 Mg implantation or supplementation ameliorates bone disorder in CFTR-mutant mice (ΔF508) through an ATF4-dependent Wnt/β-catenin signalling

Magnesium metal and its alloys are being developed as effective orthopaedic implants; however, the mechanisms underlying the actions of magnesium on bones remain unclear. Cystic fibrosis, the most common genetic disease in Caucasians caused by the mutation of CFTR, has shown bone disorder as a key clinical manifestation, which currently lacks effective therapeutic options. Here we report that implantation of magnesium-containing implant stimulates bone formation and improves bone fracture healing in CFTR-mutant mice. Wnt/β-catenin signalling in the bone is enhanced by the magnesium implant, and inhibition of Wnt/β-catenin by iCRT14 blocks the magnesium implant to improve fracture healing in CFTR-mutant mice. We further demonstrate that magnesium ion enters osteocytes, increases intracellular cAMP level and activates ATF4, a key transcription factor known to regulate Wnt/β-catenin signalling. In vivo knockdown of ATF4 abolishes the magnesium implant-activated β-catenin in bones and reverses the improved-fracture healing in CFTR-mutant mice. In addition, oral supplementation of magnesium activates ATF4 and β-catenin as well as enhances bone volume and density in CFTR-mutant mice. Together, these results show that magnesium implantation or supplementation may serve as a potential anabolic therapy for cystic fibrosis-related bone disease. Activation of ATF4-dependent Wnt/β-catenin signalling in osteocytes is identified as a previously undefined mechanism underlying the beneficial effect of magnesium on bone formation.

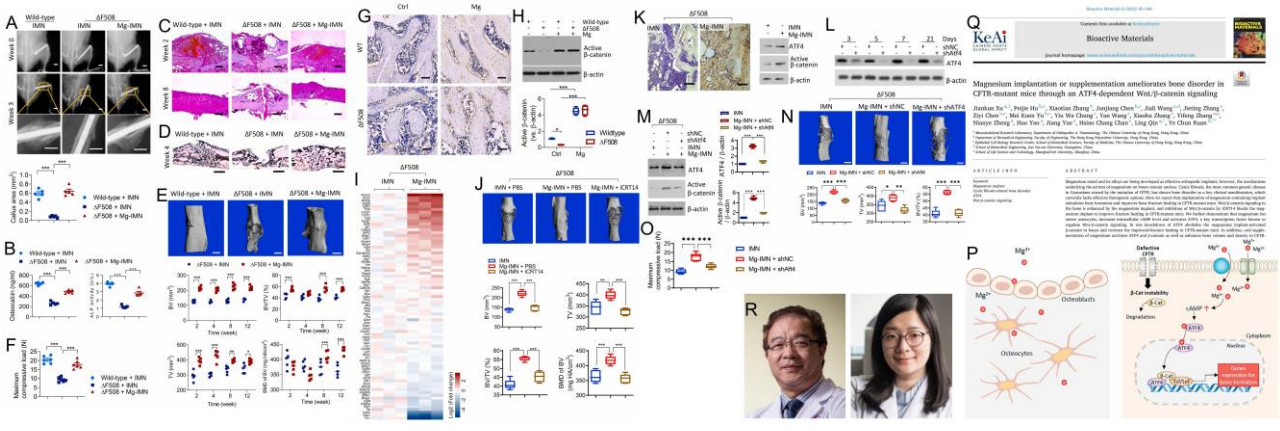

Figure 4. Mg implantation or supplementation ameliorates bone disorder in ΔF508 mice through an ATF4-dependent Wnt/β-catenin signaling (Selected from Xu JK, et al., ***Bioactive Materials,*** Vol 8, 2022). **(A-F)** Magnesium-inserted intramedullary nail (Mg-IMN) rescues the impaired fracture healing in ΔF508 mice. **(G-J)** Magnesium implantation restores Wnt/β-catenin signaling to promote bone formation in ΔF508 mice. **(K-O)** ATF4 is required for magnesium-enhanced bone regeneration in ΔF508 mice. **(P)** Schematic diagram showing the effect of magnesium on bone formation with CFTR deficiency. Entering bone forming cells through Mg^{2+} channels or transporters, Mg^{2+} induces cAMP and the activation of transcription factors, ATF4 and β-catenin (β-Cat), rescuing CFTR deficiency-impaired Wnt/β-catenin signaling to promote bone formation. **(Q)** The cover page of this published study. **(R)** The authors from this TRS team for this study **(Prof. Ling QIN and Prof. Yechun RUAN).**

The present results have collectively shown that magnesium, by activating ATF4 and reversing the CFTR deficiency-impaired Wnt/β-catenin signalling, enhances bone formation, resulting in increased bone volume, density and strength as well as improved femoral fracture healing in bone diseases associated with CFTR deficiency. It therefore highlights Mg as a cost-effective approach with translational potential. The present study also shows successful application of magnesium-containing hybrid fixation systems in load-bearing bones. (Xu JK, et al., ***Bioactive Materials,*** Vol 8, 2022, https://doi.org/10.1016/j.bioactmat.2021.06.034)

6.1.5 Biodegradable Mg combined with distraction osteogenesis synergistically stimulates bone tissue regeneration via CGRP-FAK-VEGF signalling axis

Critical size bone defects are frequently caused by accidental trauma, oncologic surgery, and infection. Distraction osteogenesis (DO) is a useful technique to promote the repair of critical size bone defects. However, DO is usually a lengthy treatment, therefore accompanied with increased risks of complications such as infections and delayed union. Here, we demonstrated that magnesium (Mg) nail implantation into the marrow cavity degraded gradually accompanied with about 4-fold increase of new bone formation and over 5-fold of new vessel formation as compared with DO alone group in the 5 mm femoral segmental defect rat model at 2 weeks after distraction. Mg nail upregulated the expression of calcitonin gene-related peptide (CGRP) in the new bone as compared with the DO alone group. We further revealed that blockade of the sensory nerve by overdose capsaicin blunted Mg nail enhanced critical size bone defect repair during the DO process. CGRP concentration-dependently promoted endothelial cell migration and tube formation. Meanwhile, CGRP promoted the phosphorylation of focal adhesion kinase (FAK) at Y397 site and elevated the expression of vascular endothelial growth factor A (VEGFA). Moreover, inhibitor/antagonist of CGRP receptor, FAK, and VEGF receptor blocked the Mg nail stimulated vessel and bone formation. We revealed, for the first time, a CGRP-FAK-VEGF signalling axis linking sensory nerve and endothelial cells, which may be the main mechanism underlying Mg-enhanced critical size bone defect repair when combined with DO, suggesting a great potential of Mg implants in reducing DO treatment time for clinical applications.

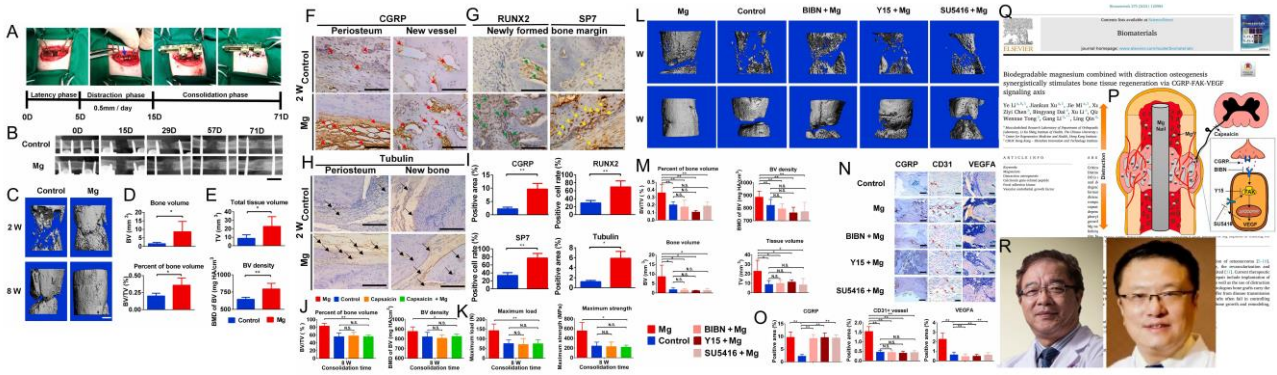

Figure 5. Biodegradable Mg combined with distraction osteogenesis synergistically stimulates bone tissue regeneration via CGRP-FAK-VEGF signaling axis (Selected from Li Y, et al., ***Biomaterials,*** Vol 275, 2021). **(A)** Surgical procedure for establishing 5 mm critical size

midshaft bone defect model. Mg nail was implanted into the femur marrow cavity in Mg group, while Control group with no implantation. **(B)** The procedure of DO. **(C)** X-ray performed at 0, 15, 29, 57, and 71 days after surgery. Scale bar: 5 mm. **(D)** Representative 3D reconstructed images showing the newly formed bone at 2 weeks and 8 weeks after distraction. Scale bar: 1 mm. **(E)** Quantitative data from 263 slides in the bone defect region at 2 weeks after distraction. **(F)** Immunohistochemical staining of CGRP (Red arrow) in newly formed tissues at 2 weeks after distraction. Scale bar: 100 μm. **(G)** Representative IHC images showed that the expression bone formation marker RUNX2 (Green arrow), and SP7 (Yellow arrow). Scale bar: 100 μm. **(H)** Representative IHC images showed that the expression bone formation marker RUNX2 (Black arrow). Scale bar: 200 μm. **(I)** Semi-quantification of CGRP, RUNX2, SP7, and Tubulin positive cell rate or area in bone defect region at 2 weeks after distraction. **(J)** Micro-CT quantitative data showing the difference in BV/TV and BMD in the newly formed bone compared among Control, Mg, Capsaicin, and Capsaicin + Mg groups at 8 weeks after distraction. BV/TV, bone volume fraction; BMD of BV, bone volume density. **(K)** The maximum load and maximum strength of the entire femur healing complex compared among Control, Mg, Capsaicin + Mg, and Capsaicin groups at 8 weeks after distraction. **(L)** Representative 3D reconstructed images showing the difference of newly formed bone in different groups at 2- and 8- weeks during the consolidation phase. **(M)** Micro-CT quantitative data showing the difference in BV, TV, BV/TV, BV BMD, and TV BMD of newly formed bone in different groups at 2 weeks after distraction. **(N–O)** Immunohistochemical staining and qualification of CGRP (Brown), CD 31 (Red arrow), and VEGFA (Brown) in the newly formed tissues at 2 weeks after distraction. **(P)** Schematic diagram showing the proposed mechanism of pure Mg nail enhanced critical size bong defect repair during DO. Mg nail implantation into the marrow cavity increased new bone and vessel formation during DO. Mg nail implantation upregulated the expression of CGRP during DO. Blockade of the sensory nerve using capsaicin blunted the Mg nail-enhanced critical size bone defect repair during DO. CGRP receptor antagonist, FAK inhibitor, and VEGFR-2 inhibitor also blunted the Mg-facilitated bone formation and vessel formation during DO. **(Q)** The cover page of this published study. **(R)** The authors from this TRS team for this study **(Prof. Ling QIN and Prof. Gang LI).**

In this study, a pure Mg nail was applied to accelerate bone formation in DO, offering a proof of concept for developing Mg-based implants to reduce consolidation time for critical size bone defect repair. Our results have proven the study hypothesis that Mg is effective for accelerating critical size bone defect repair during DO process and the CGRP-FAK-VEGF signalling axis linking sensory nerve and endothelial cells may be the main mechanism.(Li Y, et al., ***Biomaterials,*** Vol 275, 2021, https://doi.org/10.1016/j.biomaterials.2021.120984)

6.1.6 Nano-dual-phase metallic glass film enhances strength and ductility of a gradient nanograined magnesium alloy

Magnesium (Mg) alloys are good candidates for applications with requirement of energy saving, taking advantage of their low density. However, the fewer slip systems of the hexagonal – close - packed (hcp) structure restrict ductility of Mg alloys. Here, a hybrid nanostructure concept is presented by combining nano-dual-phase metallic glass (NDP-MG) and gradient nanograin structure in Mg alloys to achieve a higher yield strength (230 MPa, 31% improvement compared with the reference base alloy) and larger ductility (20%, threefold higher than the SMAT-H sample), which breaks the strength–ductility trade-off dilemma. This hybrid nanostructure is realized by surface mechanical attrition treatment (SMAT) on the surface of a crystalline Mg alloy, and followed by physical vapor deposition of a Mg-based NDP-MG. The higher strength is provided by the nanograin layer generated by SMAT. The larger ductility is a synergistic effect of multiple shear bandings and nanocrystallization of the NDP-MG, inhibition of crack propagation from the SMATed nanograined structure by the NDP-MG, and strain-induced grain growth in the SMATed nanograin layer. This hybrid nanostructure design provides a general route to render brittle alloys stronger and ductile, especially in hcp systems.

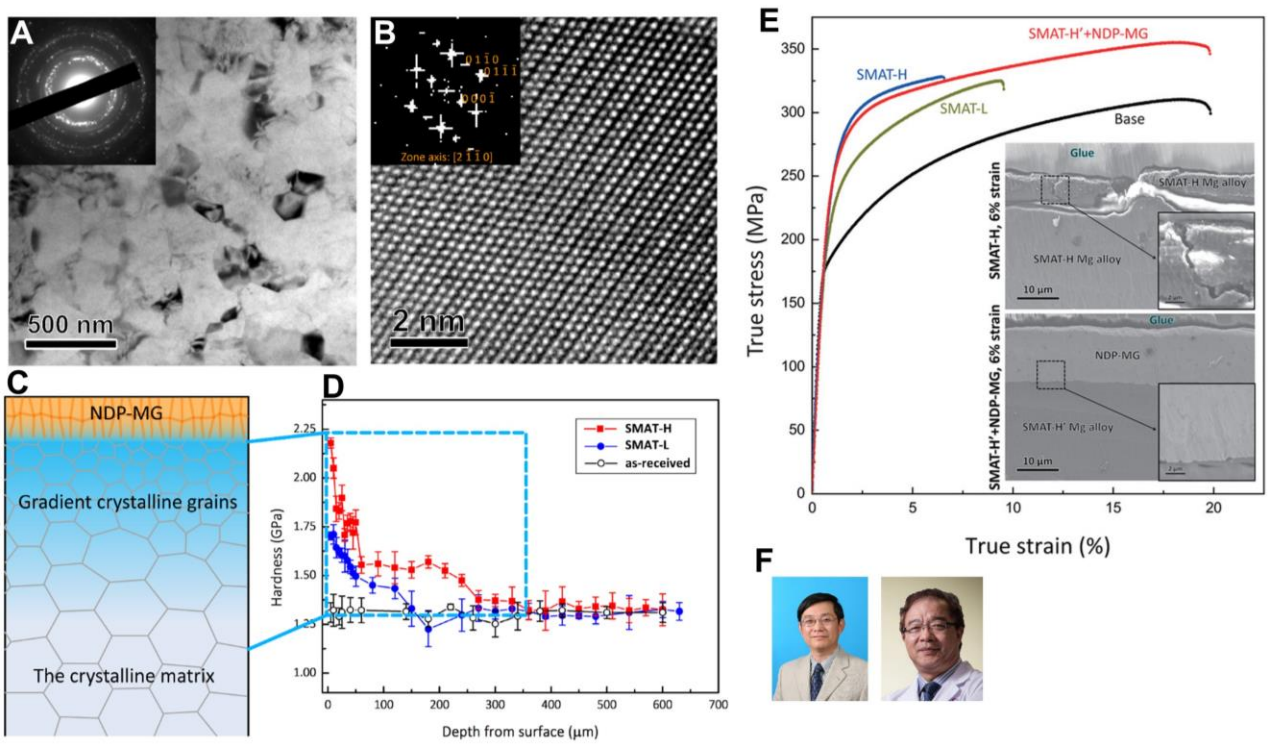

Figure 6. Gradient grain structure of the SMATed Mg alloy. A) Typical bright-field TEM image of the nanograin layer, probing from 20 μm depth from the SMAT-H Mg alloy surface. The inset shows a selected area electron diffraction (SAED) pattern. The ring feature in the SAED pattern indicates a weak crystallographic texture. B) HRTEM image of a nanograin, probing from [2 -1 -1 0] zone axis. The inset is the corresponding fast Fourier transform image. C) Schematic illustration of the hybrid nanostructure, showing NDP-MG on the top of the gradient crystalline grains, followed by the crystalline matrix in the interior. D) Hardness values measured at different depths from the surface of the as-received, SMAT-L and SMAT-H Mg alloys. E) Mechanical properties of the NDP-MG coated SMAT-H' Mg alloy at room temperature. True stress–strain curves of base (black), SMAT-L (dark yellow), SMAT-H (blue), and NDP-MG coated SMAT-H' (red) Mg alloys. The insets are cross-sectional SEM images of the SMAT-H and NDP- MG coated SMAT-H' Mg alloys with 6% true strain, showing crack propagation impeding by the NDP-MG. F) The collaborators from this TRS team for this study **(Prof. Jian LU and Prof. Ling QIN).**

We developed a hybrid nanostructured Mg alloy via depositing a Mg-based NDP-MG film on the surface of the gradient nanograined Mg alloy. The synergistic effects of multiple shear bandings and crystallization of the NDP-MG, crack impediment by the NDP-MG, and grain growth of the SMATed nanograin layer promote the large plastic deformation. These mechanisms facilitate excellent mechanical properties in Mg alloys, overcoming the strength–ductility trade-off dilemma. (Liu C., et al., ***Advanced Science,*** Vol 7, 2020, https://doi.org/10.1002/advs.202001480).

6.1.7 Bone regeneration promoted by low-intensity pulsed ultrasound (LIPUS) + PLGA/TCP + Icaritin + Secretome

Low-intensity pulsed ultrasound (LIPUS) is a developing technology, which has been proven to improve fracture healing process with minimal thermal effects. This non-invasive treatment accelerates bone formation through various molecular, biological, and biomechanical interactions with tissues and cells. Although LIPUS treatment has shown beneficial effects on different bone fracture locations, only very few studies have examined its effects on deeper bones. This study provides a new combined strategy of LIPUS and the classic PLGA/TCP, Icaritin and secretome for bone regeneration.

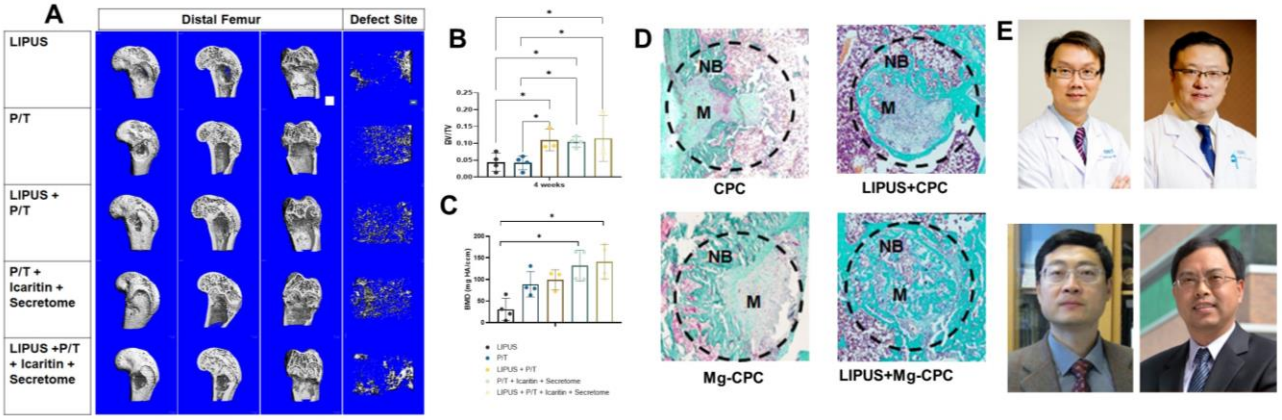

Figure 7. A) 3D reconstructed models of the left femur lateral epicondyle and the newly formed bone. The scanning resolution is 18.5 μm at 70 kV,114 μA with 211-1000 threshold. (LIPUS: OVX rats treated with LIPUS after drill hole surgery, P/T: OVX rats implanted with PLGA/TCP, LIPUS + P/T: OVX rats implanted with PLGA/TCP and treated with LIPUS after drill hole surgery, P/T+ Icaritin + Secretome: OVX rats implanted with PLGA/TCP/Icaritin + Secretome, LIPUS + P/T+ Icaritin + Secretome: OVX rats implanted with PLGA/TCP/Icaritin + Secretome and treated with LIPUS after drill hole surgery). B&C) Quantification data of BV/TV, BMD of defect site at week 4 after bone defect surgery within different threshold (158-1000 and 211-1000). Values are given as mean ± SD. (*P < 0.05, **P < 0.005, ***P < 0.001, one-way ANOVA, n=4) .**(Prof. Winghoi CHEUNG, Prof. Gang LI, Prof. Weijia LU, and Prof. Yongping ZHENG).**

This study disclosed that the compined LIPUS + PLGA/TCP + Icaritin + Secretome treatment enhanced bone regeneration, and further study will be conducted to confirm the therapeutic effect.

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 TRS funding is an important milestone for our studies as it is an important mediator between the basic studies and clinical applications. By collaboratively conducting this TRS project, we have established a Bedside to Bench to Bedside platform that link up the academic institutes, industrial partners, and the qualified third parties to provide the certificated reports that are crucial for the clinical translations such as the multi-center Randomized Clinical Trials (RCT). By successfully completing this TRS project, we not only conducted this innovative research project with R&D of Class III medical implants, but also established a translational medicine platform which allow us to continue the downstream clinical translation and product commercialization. Apart from a significant number of publications in top scientific or professional journals, our scientific based or pathophysiology of relevant disorder-oriented product development facilities the clinical translation and establishment of Area of Excellence (AoE) and Research Impact Fund (RIF) for studying musculoskeletal degeneration and innovation-based regeneration.

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

6.4 (a) Please provide details of each peer-reviewed journal publication arising directly from this project by completing the table at **Annex** *(one for each publication)*.
The publications have been filled in the table and please check.

(b) Please provide details of recognised international conference(s) in which paper(s) related to this project was/were delivered by completing the following table:
(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)*
04/2018/ Hangzhou China	Novel tendon-derived stem cell sheet secures functional repair of torn rotator cuff through TGF-β3 and IGF-1 signaling	International Symposium on Ligaments & Tendons (ISL&T XVII)	2018	No	Yes	No
05/2018/ Shanghai China	Targeting CD10+CD26+S100A4+ Fibroblasts to Ameliorate Delayed Healing of Bone-fractures	12th International Congress on Orthopaedic Advanced Technique and Clinical Translational Research (OTR)	2018	No	Yes	No
05/2018/ Hong Kong China	Magnesium Stimulates Calcitonin Gene-Related Peptide from Sensory Nerve System to Augment the Healing of Osteoporotic Bone Fracture	4th Asia-Pacific Bone & Mineral Research Meeting and Osteoporotic Fracture Prevention & Treatment Conference	2018	No	Yes	No
05/2018/ Hong Kong China	Asiatic Acid Attenuates Bone Loss by Regulating Smad7/TAK1/NF-κB Signaling Pathway in Osteoclastogenesis.	4th Asia-Pacific Bone & Mineral Research Meeting and Osteoporotic Fracture Prevention & Treatment Conference	2018	No	Yes	No
06/2018/Hong Kong	Designing high corrosion-resistant and plastic biomaterial with ultrahigh-strength (Dr. Chang LIU)	NANO 2018 XIV International Conference on Nanostructured Materials	2018	No	Yes	No
06/2018/Hong Kong	Dual-phase supra-nanostructuring as a route to near-ideal strength magnesium alloys (Dr. Ge WU)	NANO 2018 XIV International Conference on Nanostructured Materials	2018	No	Yes	No
10/2018/Suzhou	Targeting Sclerostin-expressing Fibroblasts to Ameliorate Delayed Healing in Challenging Bone-fractures	the 9th International Conference on Osteoporosis and Bone Research 2018	2018	No	Yes	No
10/2018/Suzhou	Bone Fragments Fixation With Microarc Oxidation-Treated Magnesium Pins Enhanced Healing Of Patellar Fracture In Rabbits	the 9th International Conference on Osteoporosis and Bone Research 2018	2018	No	Yes	No

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)*
10/2018/Suzhou	Blockage of Smad3 signaling Enhances Rotator Cuff Repair through Reducing the Aberrantly Distributed Sclerostin-producing Fibroblasts	the 9th International Conference on Osteoporosis and Bone Research 2018	2018	No	Yes	No
11/2018/Xiamen	Previously Unrecognized Role of Fibroblasts in Delayed Healing of Bone-fractures	The 13th International Congress of Chinese Orthopaedic Association	2018	No	Yes	No
11/2018/Xiamen	Small Molecule SIS-3 Improves Rotator Cuff Repair via Inhibiting the Expression of Sclerostin in a New Subpopulation of Fibroblasts	The 13th International Congress of Chinese Orthopaedic Association	2018	No	Yes	No
04/2019/Seattle	Photo-crosslinked GelMA hydrogel as drug carriers to locally applicate abaloparatide in promoting bone defect regeneration	Society for Biomaterials 2019 Annual Meeting and Exposition	2020	No	No	No
July 15, 2019, Shenzhen, China	Involvement of cystic fibrosis transmembrane conductance regulator in age-related bone disorder	The 4th International Chinese Musculoskeletal Research Conference	2020	No	Yes	No
Oct 8 2022	Magnesium-encapsulated injectable hydrogel and 3D-engineered polycaprolactone conduit facilitate peripheral nerve regeneration	Tissue Engineering and Regenerative Medicine International Society - Asia-Pacific (TERMIS-AP), Southern Korea	2022	No	Yes	No
Nov 10-11 2022	Designed and Translation of Osteogenic Mg-based Biodegradable Implants for Skeletal Fixation and Regeneration	NSFC-RGC Academic Symposium on Bone and Joint Degeneration and Regeneration, Hong Kong	2022	No	Yes	No
Dec 11 2022 HKU, Hong Kong	Functional Bone Regeneration in Challenging Bone Disorders and Defects	Theme-based Research Scheme Public Symposium 2022	2022	No	Yes	No

Due do COVID-19, no physical conferences have been organized by the members of this grant. Invitations as speakers in virtual meetings have been made for our members.

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)*
June/2020	The B-B-B Journey of Biodegradable Magnesium in Orthopaedics	ICMRS Webinar	N/A Invited presentation	No	Yes	No (https://www.icmrs.net/event-3852303)
Aug/2020	A Platform for Publishing Translational Research in ICMRS Society Journal JOT	ICMRS Webinar	N/A Invited presentation	No	Yes	No (https://www.icmrs.net/event-3930090)
July/2020	Design and Translation of Osteogenic Mg-based Biodegradable Implants for Skeletal Fixation and Regeneration	Shanghai Tech LIFE Research Seminar of Shanghai University	N/A Invited presentation	No	Yes	No
July/2020	Opening Remarks on the Important of Collaboration in Medical Research in Grater Bay Areas	2020 Medical Doctor Association Annual Meeting, Guangzhou	N/A Invited presentation	No	Yes	No
Aug/2020	Fracture Healing Mechanism Based Design of Mg-containing Hybrid Fixation Implants	The 10th Implantable Interventional Medical Device Innovation Summit 2020	N/A Invited presentation	No	Yes	No
Dec 2020	Design and Clinical Translation of Mg-based Biometal for Orthopaedic Applications	2020 Chongqing Yuelei International Design Forum	N/A Invited presentation	No	Yes	No
Feb/2021	Update of R&D of Mg-based Biodegradable Implants and Its Clinical Translation in Orthopaedics	Biomaterials Translational Forum 2021-02	N/A Invited presentation	No	Yes	No
May/2021	中药防治骨坏死的研究	"骨保人生"-骨坏死多学科中西对话论坛	N/A Invited presentation	No	Yes	No
Oct/2021	天然矿植物骨质疏松防治和促进骨折愈合的基础和临床转化研究	西安国际骨科学术会议	N/A Invited presentation	No	Yes	No
Nov/2021	天然矿植物骨质疏松防治和促进骨折愈合的基础和临床转化研究	第十次“铁代谢与骨代谢相关研究”国家级继教班	N/A Invited presentation	No	Yes	No
Dec/2021	天然矿植物骨质疏松防治和促进骨折愈合的基础和临床转化研究	北京协和代谢性骨病临床研修班暨骨质疏松症高峰论坛	N/A Invited presentation	No	Yes	No
Mar/2022	3D printing and tissue engineering for challenging musculoskeletal regeneration	the 5th ARI-SYSU Webinar	N/A Invited presentation	No	Yes	No
Apr/2022	R&D and Clinical Applications of 3D Printed Bioactive Orthopaedic Implants	2022 积水潭骨科论坛基础会场	N/A Invited presentation	No	Yes	No

(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.

N/A

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

By conducting this TRS project, we have established a platform to push forward the commercialization of our innovative Mg-based implants and other types of products, such as with Mr. H. Jiang from *Ultracare ltd.* for designing and optimizing Mg-based medical implants, and now working together on several products' clinical translation including the implant specifications and lab-scale production; with Dr. X. Shi for the big animal experiments, with Prof. J. Liang from *Sichuan Testing Center* for analyzing biosafety of the Mg-based implants and Mr. P Cai from *De. testing* for type-test to prepare the necessary documents for the CRO to coordinate multi-center clinical trials and Class III medical product registration under National Medical Products Administration (NMPA).

Moreover, following the the new policy *Regulations on supervision and administration of Medical Devices* (so-called *registrant system*), Our team has recently established the start-up companies *Medical Magnesium Ltd. (醫鎂醫療有限公司)* in Hong Kong, and for facilitating Class III medical implants clinical translation, we have also registered *CnMg MedTech Ltd. (華鎂醫療科技(深圳)有限公司)* in Shenzhen with licensing the right-of-use of patents from ORKTS of CUHK (CUHK still keeps the 100% ownership) as an essential requirement for engaging CRO to conduct RCT under the NMPA Class III Medical implants Registrant System. According to market volume analysis of China orthopaedic trauma from *IQVIA*, our Mg-based implants, especially the Mg-Ti screw plate system, will cover the most trauma market. We have signed an OEM (Original Equipment Manufacturer) contract with *Ultracare Limited* and will seek more appropriate cooperation with other companies to realize our commercialization plan.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Ms. Xiaoting ZHANG	PhD Student (CUHK)	August 2018	July 2022
Mr. Xuan HE	PhD Student (CUHK)	August 2017	July 2021
Miss Meng Chen Michelle LI	PhD Student (CUHK)	Aug 2018	July 2022
Miss Lizi CHENG	PhD Student (CityU)	Sept 2017	Sept 2021
Mr. Yunhu HE	PhD Student (CityU)	Sept 2018	Aug 2021
Mr. Mohammad Alamgir HOSSAIN	PhD Student (CityU)	Sept 2018	Aug 2021
Mr. Chen Min WANG	PhD Student (HKU)	Jan 2020	Jan 2024
Mr. Dzi Shing Aaron LAU	PhD Student (HKU)	Sept 2019	Sep 2021
Mr. Peijie HU	PhD Student (PolyU)	Jan 2018	Jan 2021
Mr. Xiaotian ZHANG	MPhil Student (PolyU)	Sept 2019	Aug 2021
Poornima PALANISAMY	MPhil student (PolyU)	Sep 2019	Aug 2021
Mr. Chieng Herng Ee	MBBS student (HKU)	Sept 2020	Aug 2023

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:
Based on this TRS project, we have established the annual postgraduate student training programme to educate the new students and staffs in September and October every year.

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.

N/A

Project Management

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

Apart from leading the small and large animal experiments and fabrication of the Mg-based implants, the PC leads the project meetings and meets with the Co-PIs and leaders of each sub-team to discuss the progress and problems encountered. The PC also tried to make sure each team has sufficient funding to complete their assigned tasks. The PC has also explored other collaboration opportunities to support related conferences and seminars. During the first four months of 2020, there was the outbreak of the COVID-19 that cover must of this TRS period. The PC still met with the team members via ZOOM and arrange group discussion to follow up with the progress of the project. However, the project progress was still delayed due to universities policy to work from home, so our team was unable to return to the laboratory to work on the experiments. After pandemic is over, PC is able to lead the multi-disciplinary and muti-institutional team to accelerate the progress and generated more academic output as well as start the preparation for clinical studies. The role of PC has also well reflected as corresponding authors of many SCI journal publications (Refer to the publication list in the relevant table at the end of report.

7. Awards and Recognition

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

Prof. Qin received the Area of Excellence grant in Jan 2021 (AoE/M-402/20) and the Research Impact Fund in Dec 2023 (R4034-23F) based on the results that were generated from this grant. More detailed information is in the part 9.4 for your kind reference.

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

Due to COVID-19, no physical conferences have been organized by our members. Yet, a number of invitations as invited speakers have been made for our members during our reporting period of May 2019 to April 2021, including following 6 major events from PC Prof. Ling QIN, 1) June 20, 2020, ICMRS Webinar; 2) July 3rd, 2020, Shanghai University, 3) July 3rd, 2020, Guangzhou, 4) August 18/19, The 10th Implantable Interventional Medical Device Innovation Summit 2020, 5) December 8th, 2020 Design-driven Innovation of Industry. 6) Feb 7th, 2021, Biomaterials Translational Forum 2021-02, 7) Oct 8 2022, Tissue Engineering and Regenerative Medicine International Society - Asia-Pacific (TERMIS-AP), Southern Korea, 8)

Nov 10-11 2022, NSFC-RGC Academic Symposium on Bone and Joint Degeneration and Regeneration, Hong Kong, 9) Dec 4 2022, Biomaterials Translational Forum: Skeletal Interoception Regulation in Bone Homeostasis and Disorders, 10) Dec 11 2022, Theme-based Research Scheme Public Symposium, Hong Kong, 11) Feb 12-15 2023, International Invention Fair in the Middle East.

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

PC Prof. Ling QIN is one of the Editors in Chief in the *Journal of Orthopaedic Translation*. This journal has an impact factor of 5.9 in 2023 and CiteScore 9.3 (2022) and 11.8 (2023), and the Top4 journal in orthopaedic research area that focus on the translational medicine. Prof. QIN is direct of the CUHK Hong Kong-Shenzhen Innovation and Technology Research Institute (Futian) since April 2020, and Prof. QIN has also been selected and foreign member of Academia Euryopia (MAE) in July 2023.

Co-PI Prof. Patrick S.H. YUNG is one of the editors-in-Chief of the *Asia-Pacific Journal of Sports Medicine, Arthroscopy, Rehabilitation and Technology*. This journal has an impact factor of 2.05 in 2023.

Co-PI Prof. Jian LU is the Academician of National Academy of Technologies of France; French Knight Order of National Merit (Chevalier de l'Ordre National du Mérite) of French Knight of the National Order of Légion d'Honneur (Chevalier de la Legion D'honneur); Senior Fellow of Hong Kong Institute for Advanced Study; Director of Hong Kong Branch of National Precious Metals Material Engineering Research Center; Director of Centre for Advanced Structural Materials

Co-PI Prof. Gang Li is the Vice Chairman, Branch of bone transverse transport in diabetic foot management group, Chinese Association of Orthopaedic Surgeons, China; Vice Chairman, Branch of Regenerative Biomaterials, Chinese Association of Materials, China; Fellow of American Orthopaedic Research Society; Vice Chairman of the Guangdong-Hong Kong-Macao Orthopedics Professional Committee, Guangdong Biomedical Engineering Society and Vice-Chairman of the Guangdong-Hong Kong-Macao Greater Bay Area Orthopedics Alliance; Tissue Engineering and Regenerative Medicine International Society (TERMIS) Asia Pacific (AP) Council Member and Ambassador to TERIMPS-Global.

Co-PI Prof. Louis Cheung is the International Review Panel Member of European Cells & Materials Journal (eCM); Associate Editor of the BMC Musculoskeletal Disorders; Section Membership Committee Co-chair of the Orthopaedic Research Society (ORS) International Section of Fracture Repair Research Section and Invited reviewer for ORS annual meeting (Trauma Section).

Co-PI Prof. Yechun RUAN is the Associate editor of the Frontiers in Physiology (since 2019), and Frontiers in Endocrinology (since 2019).

Co-I Prof. Liming BIAN is the editorial board member of Biomedical Engineering Communications, and editorial board member of Materials Research and Development.

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

N/A

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

7.5.1 AO Berton Rahn Research Award 2020, Prof. Ling Qin. Rapid prototyping of custom-made bone forming tissue engineering constructs & Projects on 3D printing in collaboration with ARI consortia.

7.5.2 Gold Medal, the 47th International Exhibition of Inventions of Geneva 2022, Prof. Ling Qin, et al., Biodegradable magnesium-based hybrid implant.

7.5.3 Silver Medal, the 47th International Exhibition of Inventions of Geneva 2022, Prof. Ling

Qin, et al., Novel therapeutic device for prevention and treatment of knee osteoarthritis and related degenerative diseases.
7.5.4 Gold Medal, the 13th International Invention Fair in the Middle East 2023, Prof. Ling Qin, Dr. Ning TANG, et al., Biodegradable magnesium-based hybrid orthopaedic implants.

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.)?

Our current research will focus on these skeletal disorders and injuries with limited repair or healing potential, including osteoporotic fracture, avascular osteoneocrosis (AVN) around joints with extremely high incidence of OA, and tendon-bone insertion reconstruction. Substantial costs are involved in surgeries and subsequent rehabilitations for these severe musculoskeletal conditions and injuries. These costs impose huge socioeconomic and healthcare burden to the patient, family, healthcare system, and society in Hong Kong and worldwide. Therefore, our research focuses on enhancing healing of these skeletal disorders or injuries by augmenting the regenerative potential for our patients. This would shorten the hospital stay of the patients and thus reducing the healthcare cost to the patients and their family. For technology transfer, the patents that are related to biodegradable magnesium orthopaedic implants have been acquired by Hong Kong Company (CN Innovations), and we are continuing our collaboration with CN Innovations to develop new technology and implant design for our Mg-based implants.

Prof. Qin has also been appointed as the Director of the CUHK Hong Kong-Shenzhen Innovation and Technology Research Institute (Futian), and the Translational European Asian Network (TEA-NET) Asia Executive Director. These opportunities allow our research to gain more exposure and may attract more potential collaborator and industrial partners.

8.2 Others (please specify):

N/A

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?
Yes, our collaborative team have developed many novel products from this TRS project and generated many new ideas during the joint meeting and discussions. We will further conduct the relevant works to explore the new R&D work on both academic and industrial levels.

9.2 Whether there are new projects evolved directly from this project?
Based on the work outputs from this TRS project, Prof. Qin received the Area of Excellence grant in Jan 2021 (AoE/M-402/20) and the Research Impact Fund in Dec 2023 (R4034-23F).

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

One of the breakthroughs in clinical approval of Mg-based orthopaedic implants is that on July 1st 2019, the NMPA (formerly known as the Chinese Food and Drug Association (CFDA)) had approved a multi-centre clinical trial on the application of our high purity Mg screws on the fixation of the bone flap, with PI of the current TRS project as one of the core members in this R&D. This is

a very favourable step for our R&D proposed in the current TRS project in terms of rolling out the biosafety concerns using innovative biodegradable Mg-based bimetals for orthopaedic applications.

Prof. Ling Qin has contacted Dr. Yue DING from Sun Yat-sen University to be one of the testing centers / hospitals for conducting the clinical trial on the Mg-based hybrid interference screw for anterior cruciate ligament (ACL) reconstruction **(Figure 9.3.1 & 9.3.2)**. Since our clinical trial will involve multiple hospitals, we will need to get approval from the ethics committee of all the hospitals that we are planning to conduct the trials. To facilitate the administrative process and requirement of the NMPA, we will need to hire a Contract Research Organization (CRO) as specified in our AoE project (the following project generated directly from this TRS project) to ensure all our documents fulfil the requirement of NMPA. Before the commencement of the clinical trial, the tasks of the CRO before conducting the clinical trial include: 1. Contact the NMPA about our clinical study, 2. Draft or modify or supplement our protocol design if needed, 3. Get ethics approval from all hospitals for NMPA endorsement for commencing the multi-center clinical trials. We have drafted a clinical trial protocol for seeking Co-PIs' hospitals for obtaining their approval.

中山大学孙逸仙纪念医院伦理委员会审批文

[2020]伦申第（457）号

骨外科：

你科丁悦教授参与申请的项目“Aging, Skeletal Degeneration and Regeneration”中负责涉及的临床研究“Innovative Mg-based Interference Screw Developed for Anterior Cruciate Ligament Reconstruction as Class III Medical Implants”经医学伦理委员会审查，认为该研究基本符合医学伦理学要求，同意该临床研究的申报。

中山大学孙逸仙纪念医院
医学伦理委员会
2020 年 11 月 20 日

For funding to support this clinical trial, we have secured an AoE grant which a large portion of this grant will be used for conducting the clinical trial on ACL reconstruction using our Mg interference screw. Currently, this multi-center clinical trial is planned to commence in Q4 of 2024, as agreed by our collaborators and funding body.

Figure 9.3.1 The tentative approval applied for a multicenter clinical trial on the ACL reconstruction with Dr. DING as one of the co-principal investigators of our TRS proposal.

Sun Yat-sen Memorial Hospital
Sun Yat-sen University
107 West Yan Jiang Rd.
Guangzhou 510120
P.R. China

Professor Ling QIN
Director of Musculoskeletal Research Laboratory
Department of Orthopaedics and Traumatology
The Chinese University of Hong Kong, Hong Kong SAR, PR China

Dear Professor Qin,

November 19th, 2020

Reference: Letter of Collaboration as Principal Investigator for Multi-center Clinical Trial on Innovative Mg-based Interference Screw Developed for Anterior Cruciate Ligament (ACL) Reconstruction as Class III Medical Implants Registration Specified in Your Area of Excellence (AoE/M-402/20) Application in Hong Kong

Thanks for updating with me on possible shortlist as well as interview for your proposal on "Aging, Skeletal Degeneration and Regeneration" under above captioned scheme.
I am pleased to have such opportunity to work with you and your team as collaborator of your proposal. Based on our collaborative research in Mg interference screw with relevant publications using human cadaveric testing in my center and related degradation particle research, I am very pleased to serve as principle investigator to coordinate a Multi-center Clinical Trial specified in your AoE proposal on "Innovative Mg-based Interference Screw Developed for Anterior Cruciate Ligament Reconstruction as Class III Medical Implants".

I know that Mg-based hip flap fixation screw has been approved for multi-center clinical trials last year by National Medical Products Administration (NMPA) and most recently also your Mg-containing porous scaffold composite materials, so I am confident to lead the multi-central trial for your innovative Mg-based hybrid interference screw and its approval by NMPA. In addition, as I am chairing clinical trial committee of my hospital and aware of the essential clinical trial requirement, I am looking forward to approval of your AoE project and collaborate with the designated Clinical Contract Organization to grant human ethics upon the funding approval.

Looking forward to a successful application and preparation for clinical trial required for Class III medical product registration!

Yours sincerely,

Yue Ding

Professor Yue Ding (MD, PhD)
Director of Medical Engineering Research Center
Deputy Director of Department of Orthopaedic Surgery
Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou.
E-mail: dingyue36@126.com

Figure 9.3.2 Collaboration letter from Dr. Yue DING for supporting our grant application and subsequently be responsible for potential multicenter clinical trial on the ACL reconstruction.

Prof. QIN has signed a contract with an original design manufacturer or original equipment manufacturer (ODM/OEM) to facilitate product design and manufacture **(Figure 9.3.3)**. We have negotiated with several CRO companies **(Figure 9.3.4)**, and the tender process has been performed by CUHK. These works are the foundation for the CRO conducted Preparation for Multi-center clinical trials.

Figure 9.3.3 Prof. Ling QIN Signed a collaboration agreement with CEO of a listed company.

Figure 9.3.4 A zoom meeting between Prof. Ling QIN's team and a CRO company.

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.

PC Prof. Ling Qin: HK$64.889 million from RGC for the AoE Scheme (AoE/M-402/20, Aging,

Skeletal Degeneration and Regeneration), and HK$4 million from RGC for the RIF (R4034-23F, Magnesium-Based Biodegradable Implants as Innovative Class III Medical Devices: From Implant Design, Biosafety, Efficacy to Clinical Translation). These two project are now under performance to further push forward the clinical translation and commercialization of our innovative products that initiated from this TRS project.

Co-PI Prof. Gang Li: HK$1,185,400 from RGC for the GRF (14202920, Engineering the biomimetic structural and mechanical heterogeneity of cell-adaptable nanocomposite hydrogels for biomedical applications), and HK$2,268,317.50 from ITC for the ITF-PRP (PRP/050/19FX, Development of staphylococcal enterotoxins C2 (SEC2) as a drug to promote osteoporotic fracture healing). The hydrogel and drug in the above two projects were derived from this TRS project, and will be further refine these following studies.

Co-PI Prof. Yechun RUAN is now conducting two HMRF grants (Magnesium Implant Combined with CFTR Enhancer for Treatment of Age-Related Bone Fracture; Combining CFTR enhancers with low-intensity pulsed ultrasound for treatment of age-related bone fracture) to further explore the application of CFTR enhancer in bone fracture healing.

Co-I Prof. Liming BIAN's team has obtained the Technology Start-up Support Scheme for Universities (TSSSU/CUHK/20/07/) from ITC and Incu-Bio from Hong Kong Science and Technology Parks Corporation (HKSTP) scheme to further push forward the commercialization of the hydrogel products developed from the TRS project.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	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	112	29	1	3	Type	No.
					N.A.	N.A.

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

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
N/A	N/A

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Population aging has become a major global and healthcare issue now, according to The World Social Report 2023 launched by the United Nations, by 2025, the world's aging population over 65 years old will exceed 1.6 billion, and Hong Kong will become one of the oldest areas on the world with 40.6 % of aging population over 65 years old. Population aging is accompanied by a sharp increase in the incidence of musculoskeletal diseases such as osteoporosis, fragility fracture, and osteoarthritis, which seriously affect the quality of life of patients and bring huge economic burdens to families and society. The current mainstream titanium alloy orthopaedic implants as Class III medical devices provide only mechanical support for clinical fracture surgical fixation, yet without any biological properties for healing enhancement and anti-infections. In contrast, attributed to their excellent biodegradable and bioactive properties, magnesium metal or its alloys have been proven to promote the regeneration of bone, blood vessels, and nerves and therefore accelerate fracture healing. Thus, magnesium and its alloys have great potential for clinical use especially for elder people with osteoporotic fractures. Previous studies of our group have revealed the main molecular mechanisms of magnesium promoting skeletal regeneration. Magnesium ions released by magnesium degradation *in vivo* could stimulate the sensory nerve endings of the periosteum and release and upregulate calcitonin gene-related peptide (CGRP) from dorsal root ganglions, thus promoting bone formation. These research results were highlighted by top scientific journals including ***Science*** (https://www.science.org/doi/10.1126/science.ada1187), ***Nature*** (https://www.nature.com/articles/d42473-018-00028-w)**,** and ***Nature Reviews Endocrinology*** (https://www.nature.com/articles/nrendo.2016.160) highlighting their translational clinical potential. Based on our existing research results, in collaboration with our industrial manufacturers and testing institution partners, we aim to develop Magnesium-based medical devices that could promote fracture healing as well as provide sufficient internal fixation for bone fracture. We will focus on large animal (Goat experimental model) disease models of bone fracture in this project to obtain preclinical biosafety and effectiveness data to further promote the multi-center clinical trials and Class III biomedical device registration for broadening clinical applications. Our team is committed to developing novel Magnesium-based medical implants and devices for challenging musculoskeletal disorders and promoting the translation of Magnesium metal from basic research to clinical applications, hence reducing the healthcare and economic burden associated with the population aging.