心血管研究研讨会

Stanford-China Cardiovascular Symposium

September 21-22, 2017
Li Ka Shing Center | Paul Berg Hall | Stanford, CA

Stanford Cardiovascular Institute

Sponsored in part by the Chi-Li Pao Foundation

Stanford MEDICINE

SCHEDULE: Day 1

8:30 am **Registration & Continental Breakfast**

9:00 am Opening Remarks
Joseph C. Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Simon H. Stertzer Professor of Medicine and Radiology, Stanford

9:05 am Welcome from the School of Medicine
Lloyd B. Minor, MD
Carl and Elizabeth Naumann Professor
Dean of Stanford University School of Medicine

9:10 am Welcome from Stanford Health Care
David Entwistle, MHSA
President and CEO of Stanford Health Care

9:15 am Welcome from Lucile Packard Children's Hospital
Christopher G. Dawes, MBA
President and CEO of Lucile Packard Children's Hospital

9:20 am **Keynote**

Introduction to Keynote Speaker
by Simon H. Stertzer, MD
Professor of Medicine, Emeritus, Stanford

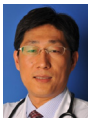
The Cardiovascular Disease Continuum: Nearly 30 Years Later
Victor J. Dzau, MD
President, National Academy of Medicine
James B. Duke Professor of Medicine, Duke University

CARDIAC SURGERY
Moderator: Anson Lee, MD
Assistant Professor of Cardiothoracic Surgery
Stanford University

9:50 am Convergence of Biologic and Surgical Reconstructive Therapies for Cardiovascular Diseases
Y. Joseph Woo, MD
Norman E. Shumway Professor
Chair, Department of Cardiothoracic Surgery, Stanford

10:00 am — Congenital Heart Disease: Pulmonary Artery Reconstruction
Frank Hanley, MD
Lawrence Crowley, MD, Endowed Professor in Child Health, Stanford

10:10 am — Heart Failure and Heart Transplant Program in Beijing Anzhen Hospital
Haibo Zhang, MD, PhD
Professor, Deputy Director, Department of Cardiac Surgery
Beijing Anzhen Hospital, Capital Medical University



10:20 am — Robotic Cardiac Surgery in China: Experience from PLA 301 Hospital
Rong Wang, MD
Associate Professor and Director, Department of Cardiovascular Surgery
Chinese PLA General Hospital

10:30 am — Panel discussion with “Cardiac Surgery” speakers

10:50 am — Coffee Break

CLINICAL SCIENCE
Moderator: Stephen Roth, MD
Professor of Pediatrics (Cardiology)
Lucile Packard Children’s Hospital

11:10 am — Prenatal and Postpartum Integration Management in China
Yihua He, MD, PhD
Professor and Chair, Consultation Center for Maternal-Fetal Medicine in Fetal Heart Disease, Beijing Anzhen Hospital No. 2

11:20 am — Heart Rhythm Control: The Original Works of Fuwai
Yan Yao, MD, PhD
Vice Director, Arrhythmia Center of Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

11:30 am — The Future of Arrhythmia Therapy
Paul Wang, MD
Professor of Medicine (Cardiovascular Medicine), Stanford
Editor of ‘Circulation: Arrhythmia & Electrophysiology’

11:40 am — Panel discussion with “Clinical Science” speakers

12:00 p.m. Networking Lunch and Poster Viewing

1:00 pm Keynote

Introduction to Keynote Speaker
by Li Wang, MD, PhD
Professor, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

The Unceasing Battle against Cardiovascular Diseases in China: Fuwai's Mission Now and Beyond
Zhe Zheng, MD, PhD
Professor, Deputy Director, National Center for Cardiovascular Diseases
Vice President, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

VASCULAR SURGERY/PERIPHERAL ARTERIAL DISEASE
Moderator: Nicholas Leeper, MD
Associate Professor of Surgery, Vascular Surgery and Medicine
Stanford University

1:25 pm Aortic Aneurysm Pathogenesis
Ronald L. Dalman, MD
Chief, Division of Vascular Surgery
Walter Clifford Chidester and Elsa Rooney Chidester Professor of Surgery, Stanford

1:35 pm The Management of Aortic Arch Disease: Open, Hybrid or Endo
Chang Shu, MD, PhD
Professor & Director, Department of Vascular Surgery, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

1:45 pm Present Situation of Chinese Treatment of Type A Acute Aortic Dissection/Overview of Anzhen
Ming Gong, MD, PhD
Associate Professor, Beijing Anzhen Hospital, Capital Medical University

1:55 pm Recent Advances in Small Diameter Blood Vessel Regeneration
Deling Kong, PhD
Professor & Dean of College of Life Sciences, Nankai University

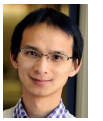
2:05 pm Panel discussion with "Vascular Surgery/Peripheral Arterial Disease" speakers

2:25 pm Coffee Break

Basic Science
Moderator: Xin-Liang 'Xin' Ma, MD, PhD
Professor, Medicine and Emergency Medicine
Director, Cardiovascular Research Program, Thomas Jefferson University

2:35 pm Uncovering the Roles of miRNA in Cardiovascular Physiology and Diseases
Dao Wen Wang, MD, PhD
Professor & Director, Department of Internal Medicine, Tongji Hospital
Huazhong University of Science and Technology

2:45 pm Single Cell Profiling Identifies INO80 as a Critical Chromatin Remodeler Dictating Cardiac Lineage Commitment
Li Wang, MD, PhD
Professor, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences



2:55 pm Development of the Right Sided Heart and Cardiac Regeneration
Zhongzhou Yang, PhD
Professor, Model Animal Research Center, Nanjing University

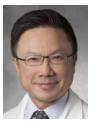
3:05 pm Applying Systems Immunology Methods to Understand Cardiovascular Health and Disease
Mark M. Davis, PhD
Director, Stanford Institute of Immunity, Transplantation, and Infection
The Burt and Marion Avery Family Professor, Stanford

3:15 pm Panel discussion with "Basic Science" speakers

3:35 pm Break

Interventional Cardiology
Moderator: David Lee, MD
Associate Professor, Cardiovascular Medicine
Stanford University

3:55 pm Transcatheter Valve Therapy: Current and Future
Alan Yeung, MD
Li Ka Shing Professor in Cardiology
Chief, Cardiovascular Medicine (Clinical), Stanford



4:05 pm

Can We Predict Myocardial Infarction: The Ongoing Search for the 'Vulnerable Plaque'

William Fearon, MD
Professor of Medicine, Cardiovascular Medicine
Director, Interventional Cardiology, Stanford

4:15 pm

Interventional Therapy in Atherosclerosis

Yida Tang, MD, PhD
Professor & Director, Department of Internal Medicine, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

4:25 pm

Panel discussion with "Interventional Cardiology" speakers

4:45 pm

Introduction to the Chinese Edition of the NEJM (医学前沿)

Rui-Ping Xiao, MD, PhD
Professor at the Institute of Molecular Medicine
Peking University

4:55 pm

Adjourn

SCHEDULE: Day 2

8:30 am

Continental Breakfast

8:55 am

Introduction to Chi-Li Pao Foundation Programs

Gloria Kim, MD
Clinical Assistant Professor, Medicine (Cardiovascular Medicine)
Medical Director, International Medical Services, Stanford Health Care

9:00 am

Keynote

Introduction to Keynote Speaker

by Daniel Bernstein, MD
Alfred Woodley Salter and Mabel Smith Salter Endowed Professor in Pediatrics, Stanford

Identification of Novel Allosteric Drugs for G Protein Coupled Receptors

Brian Kobilka, MD
2012 Nobel Laureate
Helene Irwin Fagan Chair in Cardiology and Professor of Molecular & Cellular Physiology, Stanford

CLINICAL TRIALS, INNOVATION & PARTNERSHIPS
Moderator: Kenneth Mahaffey, MD
Professor of Medicine (Cardiovascular Medicine)
Vice Chair of Clinical Research, Stanford

9:25 am — Surgery for End-Stage Heart Failure: Practice and Experience in Fuwai Hospital
Zhe Zheng, MD, PhD
Vice President, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Sciences
Professor & Deputy Director, National Center for Cardiovascular Diseases

9:35 am — Atrial Fibrillation: Personalizing Care and Improving Quality Through Global Programs
Mintu Turakhia, MD, MAS
Associate Professor of Medicine, Cardiovascular Medicine and by courtesy, of Health and Research Policy, Veterans Affairs Health Care System
Senior Director of Research and Innovation

9:45 am — Academia–Industry Partnerships
Robert A. Harrington, MD
Arthur L. Bloomfield Professor of Medicine
Chair Stanford Department of Medicine

9:55 am — Panel discussion with “Clinical Trials, Innovation & Partnerships” speakers

10:15 — Coffee Break

IMAGING
Moderator: Phillip Yang, MD
Associate Professor of Medicine (Cardiovascular Medicine)
Stanford University

10:25 am — Clinical Need and Future of Cardiac Imaging
Bin Lu, MD, PhD
Professor & Director, Department of Radiology, Fuwai Hospital
Peking Union Medical College & Chinese Academy of Medical Sciences

10:35 am — Comprehensive Cardiac CT Strategies for Coronary Disease Management
Koen Nieman, MD, PhD
Associate Professor, Cardiovascular Medicine, Stanford

10:45 am

Multimodality Molecular Imaging of Progressive Atherosclerosis

Feng Cao, MD, PhD
Professor & Deputy Director, Department of Cardiology
Chinese PLA General Hospital

10:55 am

Panel discussion with “Imaging” speakers

11:15 am

Networking Lunch and Poster Viewing

1:00 pm

Keynote

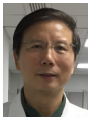
Introduction of Keynote Speaker
by Mark Mercola, PhD
Professor, Cardiovascular Medicine, Stanford

Converged Cardiac Cell Death Signaling: A Tale of CaMKII
Rui-Ping Xiao, MD, PhD
Professor at the Institute of Molecular Medicine, Peking University, Beijing, China

STEM CELLS & CARDIAC REGENERATION
Moderator: Jianyi ‘Jay’ Zhang, MD, PhD
Chair, Department of Biomedical Engineering, University of Alabama, Birmingham

1:30 pm

Overview of Translational Stem Cell Research for Heart Failure at Zhejiang Heart Center
Hong Yu, PhD, FAHA
Professor, Key Lab for Cardiovascular Diseases, The 2nd Affiliated Hospital
Zhejiang University School of Medicine



1:40 pm

Progressing in Pluripotent Stem Cell-derived Cardiac Lineage Cells
Huangtian Yang, PhD
Professor, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences

1:50 pm — The Long and the Short of It: Telomere Lengths Predict Cardiac Failure
Helen Blau, MD
The Donald E. and Delia B. Baxter Foundation Professor, Stanford

2:00 pm — Critical Regulations of Niche on Stem Cell Fate and Cardiac Regeneration
Xi-Yong Yu, MD
Professor & Dean of the School of Pharmaceutical Sciences
Guangzhou Medical University

2:10 pm — Stem Cells and Noncoding RNAs in Cardiovascular Diseases
Shijun Hu, PhD
Professor & Deputy Director, Institute for Cardiovascular Science
First Affiliated Hospital, Soochow University

2:20 pm — Panel discussion with “Stem Cells & Cardiac Regeneration” speakers

2:40 pm — Coffee Break

GENOMICS & POPULATION GENETICS
Moderator: Joshua Knowles, MD, PhD
Assistant Professor of Medicine (Cardiovascular)
Stanford University

3:05 pm — Genetic Mechanisms of Coronary Artery Disease
Thomas Quertermous, MD
William G. Irwin Professor
Chief of Cardiovascular Medicine (Research), Stanford

3:15 pm — Allele Specific Genome Editing for Inherited Diseases
Feng Lan, PhD
Professor, Deputy Director, Beijing Lab for Cardiovascular Precision Medicine
Beijing Anzhen Hospital, Capital Medical University

3:25 pm — Cardiac iPSC Biobank to Study Cardiovascular Diseases
Joseph C. Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Simon H. Stertzer Professor of Medicine and Radiology, Stanford

3:35 pm	Million Veterans Program, Genomic Sequencing Project **Philip S. Tsao, PhD** Professor of Medicine & Associate Chief of Staff (Research) Veterans Administration Palo Alto Health Care System	
3:45 pm	Opportunities and Challenges for Population and Medical Genomics in the Personal Genome Era **Carlos Bustamante, PhD** Professor and Chair, Department of Biomedical Data Science, Stanford	
3:55 pm	Panel discussion with “Genomics & Population Genetics” speakers	
4:15 pm	Closing Comments	
4:30 pm	Reception, Research Poster Viewing & Awards Judges: Sean Wu MD PhD, Mark Mercola PhD, Patricia Nguyen MD, Francois Haddad, MD	
5:30 pm	Adjourn	

ABSTRACTS

1. Right Heart End-Systolic Remodeling Index Strongly Predicts Outcomes in Pulmonary Arterial Hypertension: Comparison With Validated Models

Authors: Myriam Amsallem MD MS[a,b,] Andrew J. Sweatt MD[c], Marie C. Aymami MD[a,b], Tatiana Kuznetsova MD PhD[d], Mona Selej MD[a], HongQuan Lu MD[a,b], Olaf Mercier MD[e], Elie Fadel MD[e], Ingela Schnittger MD[a,b], Michael V. McConnell MD MSEE[a,b], Marlene Rabinovitch MD[f,g], Roham T. Zamanian MD[c,f*], Francois Haddad MD[a,b,e,f *]

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d Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium
e Division of Cardiothoracic Surgery, Marie Lannelongue Hospital, Le Plessis Robinson, France
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g Division of Pediatrics, Stanford University, Stanford, CA, USA
*Both senior authors contributed equally to this study.

Right ventricular (RV) end-systolic dimensions provide information on both size and function. We investigated whether an internally scaled index of end-systolic dimension is incremental to prognostic scores in pulmonary arterial hypertension. Methods and Results: From 2005 to 2014, 228 patients with pulmonary arterial hypertension were prospectively enrolled. RV end-systolic remodeling index (RVESRI) was defined by lateral length divided by septal height. The incremental values of RV free wall longitudinal strain and RVESRI to risk scores were determined. Mean age was 49±14 years, 78% were female, 33% had connective tissue disease, 52% were in New York Heart Association class ≥III, and mean pulmonary vascular resistance was 11.2±6.4 WU. RVESRI and right atrial area were strongly connected to other right heart metrics. Three zones of adaptation (adapted, maladapted, and severely maladapted) were identified based on the RVESRI to RV systolic pressure relationship. During a mean follow-up of 3.9±2.4 years, the primary end point of death, transplant, or admission for heart failure was reached in 88 patients. RVESRI was incremental to risk prediction scores, including the REVEAL score, the Pulmonary Hypertension Connection equation, and the Mayo Clinic model. Using multivariable analysis, New York Heart Association class III/IV, RVESRI and log NT-proBNP (N-Terminal Pro-B-Type Natriuretic Peptide) were retained ($\chi 2$, 62.2; $P<0.0001$). Changes in RVESRI at 1 year (n=203) were predictive of outcome; patients initiated on prostanoid therapy showed the greatest improvement in RVESRI. Among metrics, RVESRI demonstrated the best test–retest characteristics. Conclusions: RVESRI is a simple reproducible prognostic marker in patients with pulmonary arterial hypertension.

2. Complementary Value of Heart Failure Risk Scores on Long-term Mortality in Patients Hospitalized with Acute Heart Failure Preserved Ejection Fraction

Authors: Kalyani A Boralkar[1], Yukari Kobayashi[1], Vedant S Pargaonkar[1], Kegan J Moneghetti[1], Mirela Tuzovic[1], Gomathi Krishnan[1], Sara Bouajila[1], Matthew Wheeler[1], Dipanjan Banerjee[1], Benjamin D. Horne[2], Kirk U Knowlton[2], Paul A. Heidenreich[1], Francois Haddad[1]
1. Stanford University School of Medicine, Stanford, CA
2. Intermountain Medical Center Heart Institute, Salt Lake City, Utah

Introduction: Heart Failure preserved Ejection Fraction (HFpEF) is a major burden on the healthcare system and better understanding risk prediction in this population may help guide management. Our objective was to assess the complementary value of existing heart failure (HF) risk scores, Get with The Guidelines (GWTG), Intermountain Risk Score (IMRS) and the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) Scores

in an independent cohort of patients hospitalized with acute HFpEF. Methods: Using Stanford Translational Research Integrated Database Environment and individual chart review, we identified 580 adult patients hospitalized with acute heart failure and left ventricular ejection fraction ≥ 50%. We excluded patients with advanced liver/kidney disease, valve replacement, transplantation, active malignancy, pulmonary arterial hypertension and hypertrophic cardiomyopathy. A sub-group of 341 patients had NT-proBNP levels available. Mortality status was determined using United States Social Security Death Index and chart review. Cox proportional hazard analysis was used to determine the HF risk scores predictive of all-cause mortality in HFpEF. Results: The mean age was 76 ± 15 years with 334 (57.6%) females. Median length of hospital stay was 4 (IQR 2 – 9) days and follow up of 2.0 (IQR 0.2 – 4.6) years with a total of 140 (24.1%) deaths. On multivariate analysis, the GWTG, IMRS and MAGGIC scores were independent correlates of all-cause mortality with normalized hazard ratios of 1.48 (1.17 – 1.86), 1.41 (1.15 – 1.76) and 1.31 (1.04 – 1.70) respectively. In patients with NT-proBNP collected, NT-proBNP remained significantly associated with mortality with normalized hazard ratio of 1.52 (1.22 – 1.91) even after adjustment for the risk scores. Conclusion: In patients hospitalized with acute HFpEF, established heart failure Risk scores together with NT-proBNP can play a complementary role in outcome prediction.

3. Telomere Shortening as a Hallmark of Lethal Dilated Cardiomyopathy

Alex C Chang[1,4], John W Day[2], Joseph C Wu[3,4], Helen M Blau[1,4]
1. Baxter Laboratory for Stem Cell Biology, Microbiology and Immunology, Stanford, CA,
2. Department of Neurology, Medicine, Stanford, CA
3. Division of Cardiology, Medicine, Stanford, CA
4. Stanford Cardiovascular Institute, Medicine, Stanford, CA

Introduction: Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive disease that results from mutations in the dystrophin gene and is the most common myopathic disease in humans. Dystrophin is crucial for the formation of a dystrophin-glycoprotein complex, which connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix in both skeletal and cardiac muscles. In the heart, a loss of dystrophin leads to increased fibrosis and death in the third decade of life due to dilated cardiomyopathy. A conundrum in studying and developing therapies for DMD has been the lack of a mouse model that fully recapitulates the clinical phenotype. When we 'humanized' the mdx mouse by creating a novel mouse model with shortened telomere lengths, both the skeletal muscle and cardiac muscle phenotypes of DMD culminating in lethal dilated cardiomyopathy were fully recapitulated. Moreover, we found that the cardiomyocytes in the hearts of four patients with DMD had telomeres with 50% the length of unaffected controls. Notably, telomere lengths in smooth muscle cells within the same cardiac tissues are unaffected, serving as robust internal controls for the specificity of telomere shortening to those cells requiring dystrophin for function. Hypothesis: We hypothesize that contractile defects due to dystrophin deficiency drive telomere shortening and result in metabolic compromise and cell death due to inhibition of mitochondrial function and biogenesis. Method: To study telomere shortening in disease progression, we generated three DMD human induced pluripotent stem cell lines and differentiated them into beating cardiomyocytes (hiPSC-CMs). Results: Our data reveal progressive telomere shortening in non-dividing hiPSC-CMs that leads to a marked DNA damage response culminating in mitochondrial dysfunction. Conclusions: Patient hiPSC-CMs recapitulate in 30 days the telomere shortening that occurs in 30 years of life in DMD patients and this technology enables the study of cause and effect and tests of interventions.

4. Diagnostic Performance of Resting Distal to Aortic Coronary Pressure Using Instantaneous Wave-Free Ratio as a Reference Standard

Yuhei Kobayashi, Nils P. Johnson, Frederik M. Zimmermann, Nils Witt, Colin Berry, Allen Jeremias, Bon-Kwon Koo, Giovanni Esposito, Gilles Rioufol, Seung-Jung Park, Takeshi Nishi, Dong-Hyun Choi, Keith G. Oldroyd, Emanuele Barbato, Nico H.J. Pijls, Bernard De Bruyne, William F. Fearon, MD

Background: Recently, two randomized controlled trials suggested that the instantaneous wave-free ratio (iFR), a resting coronary physiologic index is non-inferior to fractional flow reserve for guiding revascularization. The distal to aortic coronary pressure (Pd/Pa) measured at rest is another adenosine-free index widely available in the cardiac catheterization laboratory; however, little is known about the diagnostic performance of Pd/Pa using iFR as a reference standard. Methods: A total of 763 patients were prospectively enrolled from 12 institutions. iFR and Pd/Pa were measured under resting conditions. Using iFR≤0.89 as a reference standard, the diagnostic performance of Pd/Pa and its best cutoff value were assessed. Results: By independent core laboratory analysis, iFR and Pd/Pa were analyzable in 627 and 733 patients (82.2% vs. 96.1%, p<0.001), respectively. The median iFR and Pd/Pa were 0.90 (interquartile range 0.85-0.94) and 0.92 (0.88-0.95) and the two indices were highly correlated (R2 = 0.93, p<0.001, iFR = 1.31*Pd/Pa-0.31). By receiver operating characteristics curve analysis, Pd/Pa showed excellent diagnostic performance (area under the curve = 0.98, 95% confidence interval 0.97 to 0.99, p<0.001) with a best cutoff value of Pd/Pa≤0.91. The diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were 93.0%, 91.4%, 94.4%, 93.3%, and 92.7%, respectively. These results were similar in patients with acute coronary syndrome and stable angina. Conclusions: Pd/Pa was analyzable in a significantly higher number of patients than iFR. Pd/Pa showed excellent diagnostic performance against iFR and would likely result in similar outcomes as iFR, if used to guide revascularization.

5. Long-Term Prognostic Value of Invasive and Non-Invasive Measures Early after Heart Transplantation

Yukari Kobayashi, MD; Yuhei Kobayashi, MD; Hyoung-Mo Yang, MD; Helen Luikart, RN; Takeshi Nishi, MD; Dong-Hyun Choi, MD; Ingela Schnittger, MD; Hannah A. Valantine, MD; Kiran K. Kush, MD; Alan C.Y. Yeung, MD; Francois Haddad, MD; William F. Fearon MD
Stanford Cardiovascular Institute and Stanford Cardiovascular Medicine

Background: Invasively assessed coronary microvascular resistance early after heart transplantation (HT) predicts worse long-term outcome; however, little is known about the relationship between microvascular resistance, left ventricular function and outcomes in this setting. Methods: A total of 100 cardiac transplant recipients had fractional flow reserve (FFR) and the index of microcirculatory resistance (IMR) measured in the left anterior descending artery and echocardiographic assessment including left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS) at 1 year after HT. The primary endpoint was the cumulative survival free of death or retransplantation after 1 year. Results: The mean FFR, IMR, LVEF, and GLS values at 1 year were 0.87±0.06, 21.3±17.3, 60.4±5.4%, and 14.2±2.4%, respectively. FFR had no significant correlation with LVEF (p = 0.94) and GLS (p = 0.86). Similarly, IMR had no significant correlation with LVEF (p = 0.54) and GLS (p = 0.90). During a mean follow-up of 6.7±4.2 years (7.7 years after transplant), the primary endpoint occurred in 24 patients (24.0%). By ROC curve analysis, IMR = 19.3 and GLS = 13.3% were the best cutoff values for predicting death or retransplantation. The cumulative event-free survival was significantly lower in patients with higher IMR (log-rank p = 0.02) and lower GLS (log-rank p<0.001). The cumulative event-free survival could be further stratified with the combination of IMR and GLS (p<0.001). By multivariate Cox-proportional hazards model, higher IMR and lower GLS were independently associated with the long-term death or re-transplantation (elevated IMR, HR = 2.50, p = 0.04 and reduced GLS, HR = 3.79, p = 0.003). Conclusions: Invasively assessed coronary microvascular resistance (IMR) does not correlate with left ventricular systolic function (GLS) at 1 year after heart transplantation. IMR and GLS determined at 1 year are independent predictors of late death or retransplantation.

6. Single Cell Profiling Identifies INO80 as a Critical Regulator Dictating Cardiac Lineage Commitment

Yingnan Liao[1], Peng Yu, PhD[1], Zongna Ren[1] , Li Wang, MD, PhD[1]
1 State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China.
Correspondence: wangl@pumc.edu.cn

During human heart development, multiple molecular signaling pathways drive fate decisions. Here, we present a study of early human heart development using single-cell transcriptomics and a 2D in vitro heart development model. We computationally identify INO80 complex accompanied cardiogenesis and experimentally confirm the predictions. Knockdown of Ino80 represses genes that are implicated in cardiac differentiation. Ino80 localizes to cardiac specific regulatory regions, impacting cardiac differentiation gene expression. In conclusion, INO80 may alter the cardiac epigenome, instructing gene expression changes that drive cardiac differentiation.

7. Spatially Patterned Atheroprotective Vascular Grafts for Enhanced Patency

Karina H. Nakayama[1,2], Masashi Kawamura[2,3], Hanway Wang[1], Joseph Woo[2,3], Ngan F. Huang[1,2,3]

1 Veterans Affairs Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA, USA
2 The Stanford Cardiovascular Institute, Stanford University, Stanford, CA, USA
3 Department of Cardiothoracic Surgery, Stanford University, Stanford, CA, USA

With 25 million American suffering from at least one clinical manifestation of atherosclerosis, and approximately 500,000 deaths annually attributed to coronary artery disease, there is a pressing need to better understand the biological basis for the development of atherosclerosis. Our previous work demonstrated that spatial patterning cues from extracellular matrices (ECMs) play an important role in modulating endothelial cell (EC) angiogenic phenotype including inhibiting adhesion of lipogenic proteins and monocytes in the circulating blood that leads to atherogenesis. Therefore, we hypothesized that vascular grafts containing longitudinally oriented spatial patterning will promote aligned re-endothelialization to provide atheroprotective function and improved graft patency, when compared to vascular grafts without cell patterning. Randomly-oriented or aligned vascular grafts (0.5 cm length, 1 mm diameter) were fabricated from electrospun poly (ε-caprolactone) using a dual layer approach. Heparin was conjugated to the grafts and demonstrated a release rate of 202 μg per scaffold over a 48h period. Transplantations of heparinized acellular grafts into the rat carotid artery demonstrated patency by B-mode echo 24h post transplant. Studies are currently underway to assess long term patency and histology of grafts transplanted into the rat carotid artery. Findings from these studies will provide novel insights into the mechanisms of resisting atherogenesis that are mediated by spatial cell patterning, and have important translational potential in the generation of atheroprotective vascular grafts that enhance graft patency.

8. Clinical Outcome and Long-term Follow-up in Patients with Angina in the Absence of Obstructive Coronary Artery Disease

Authors: Vedant S Pargaonkar MD[1], Yuhei Kobayashi MD[1], Takumi Kimura MD PhD[1], Ingela Schnittger MD[1], Ian S Rogers MD MPH[1], David P Lee MD[1], William F Fearon MD[1], Alan C Yeung MD[1], Marcia L Stefanick PhD[2], Jennifer A Tremmel MD MS[1]
1. Stanford Cardiovascular Institute, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford
2. Department of Medicine, Stanford University School of Medicine, Stanford CA

Introduction: A comprehensive invasive evaluation of patients with angina in the absence of obstructive coronary artery disease (CAD) may identify occult coronary abnormalities contributing to symptoms, and may also help guide therapy. However, whether this has an impact on symptoms and quality of life (QOL) is unclear. Our objective was to study the long-term follow-up of anginal symptoms in patients with endothelial dysfunction, microvascular dysfunction (MVD), and/or a myocardial bridge (MB). Methods: Before and after invasive testing, we administered a Seattle angina questionnaire (SAQ) to 157 women and 48 men found to have no obstructive CAD on angiography. Endothelial dysfunction was defined as a decrease in luminal diameter of >20% after intracoronary (IC) acetylcholine, MVD was defined as an index of microvascular resistance ≥ 25, and an MB was defined as an echolucent half-moon sign and/or ≥10% systolic compression on intravascular ultrasound. Results: Median follow up was 1.9 (1.7 – 2.2) years. Endothelial dysfunction was present in 113 (55%) patients,

MVD in 47 (23%), and MB in 93 (45%). Medical treatment and lifestyle changes were suggested according to the diagnosis of the testing. Eleven had surgical unroofing of an MB. There were no MACE including MI, revascularization, or stroke. There were 2 deaths (both cancer). There was a significant improvement in physical limitations due to angina, anginal stability, anginal frequency, treatment satisfaction, and QOL ($p < 0.001$). On multivariable logistic regression analysis, arterial compression after IC acetylcholine, post-invasive testing calcium channel blocker usage, and age were independent predictors of significant improvement in SAQ scores. Conclusion: A comprehensive invasive evaluation can identify occult coronary abnormalities in patients with angina in the absence of obstructive CAD and help guide therapy, which ultimately results in an improvement in patients' symptoms and QOL.

9. RNASeqFPro, a Pipeline for RNA-Seq Differential Gene Expression Analysis Using Transcription Noise Model

Milos Pjanic[1,2], Clint L. Miller[1,2], Thomas Quertermous[1,2]
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RNA-Seq analysis comprises multiple steps, including quality control, mapping, read counting and differential expression (DE) analysis. Here we present a pipeline for automated RNA-Seq DE analysis based on top isoform selection. RNASeqFPro can perform analysis on paired-end or single-read RNA-Seq data for mouse mm10 and human hg19 genomes. RNASeqFPro sorts and pairs fastq files, then, performs FastQC quality control, and maps fastq files to the reference genomes using STAR second pass mapping. It uses featureCounts to perform read summarization on the GENCODE mouse or human transcript collection. It calls fileMulti2TableMod1, an awk script we developed to generate the mastertable and writes an R script to select the top transcript and converts GENCODE to RefSeq identifiers using biomaRt. Finally, it writes an R script and performs DESeq analysis of differential gene expression, creates output tables and generates graph outputs using GO term annotations and goseq package. We also created two versions that use Kallisto for fast pseudo-alignment of the reads to the GENCODE transcripts that dramatically reduced processing time. Both STAR and Kallisto versions of RNASeqFPro are based on the transcription noise model and selection of the top expressed transcript for differential gene expression (DGE) analysis, therefore considering other transcripts rising from the same locus as transcriptional noise not contributing to the effective gene expression level. RNASeqFPro performs all steps in a sequential manner, and thus significantly saves preparation time for each run. RNASeqFPro should be useful for researchers with less computational experience as it requires a single executing command.

10. Rapid remodeling of an autologous acellular conduit into an integrated neoartery

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Vascular grafts formed by decellularized matrix have shown promising functional results in early clinical trials. However, graft failures caused by poor remodeling and pro-inflammatory immune responses are still unavoidable, and the remodeling mechanisms are also unclear. Here we developed a novel decellularized autologous vascular graft (DAVG) with looser fibers, which further was implanted as an interpositional graft in the common carotid artery. The key advantages of this methodology include: constructive immune response leading to extremely high patency rates, uniform remodeling with complete infiltration of cells into the whole graft to generate a neoartery exhibiting superiorly similar structure to a native artery. Autologous stem/progenitor cells were involved in the graft remodeling. Additional preliminary in vivo studies in the minipig model

returned encouraging results. Taken together, this approach can be quickly translated to patients and will have an impact on the regeneration of autologous tube type organs in the future. Funding: This work is supported in part by the grants from National Natural Science Foundation of China (81170110/H0203 to X.Q.), National Key R&D Plan (2016YFA0101100 to N.D.), the National Institute of Health (EB012240 and HL083900 to S.L.) and California Institute for Regenerative Medicine (A clinical fellow's training grant TG2-01164 to X.Q.).

11. Ino80 Regulates Cardiac Hypertrophy and Remodeling in Response to Stress

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Cardiac hypertrophy, a complex and dynamic process involving multiple factors, leads to heart failure ultimately, but the mechanism of its development has not yet been fully elucidated. The INO80 chromatin remodeling enzyme has roles in transcription, DNA repair and replication. INO80 facilitates pluripotency gene activation in embryonic stem cell self-renewal, reprogramming, and blastocyst development. Interestingly, INO80 has also been identified as a pivotal factor in maintaining normal heart function in zebra fish. However, the role of INO80 in cardiac hypertrophy remains unclear. This project will clarify the role and mechanism INO80 in cardiac hypertrophy at cellular, animal and molecular levels. First, we will generate LentiIno80 and LentishIno80 lentivirus to infect neonatal rat cardiomyocytes which are induced by PE to investigate the role and mechanism of Ino80 in cardiac hypertrophy at the cellular level. Secondly, we will generate a Ino80 knockout mouse model and a TG mouse model with cardiac-specific Ino80 expression which would be subjected to aortic banding surgery to clarify the role and mechanism of INO80 in cardiac hypertrophy. In the third part, we will block or activate the key downstream protein of INO80 via drugs or genetic engineering to reverse the phenotype of Ino80 knockout or transgenic mice. We aim to clarify the role and mechanism of INO80 in cardiac hypertrophy to provide new molecular targets for the prevention and therapy cardiac hypertrophy.

12. Endothelial Deletion of Ino80 Disrupts Coronary Angiogenesis and Causes Congenital Heart Disease Phenotypes

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Organ size control is an important process in developmental biology that remains incompletely understood. This applies to the mammalian heart, where the determinants that support ventricle wall expansion are not fully characterized. Further knowledge could provide insight into devastating human diseases such as Left Ventricular Non-Compaction (LVNC). We found that deleting the Ino80 chromatin remodeler from endothelial cells resulted in an LVNC-like phenotype (thin compact myocardium), which correlated with defective coronary vascularization. Deletion of Ino80 individually in the two major coronary progenitors resulted in intermediate non-compaction phenotypes. In vitro, endothelial cells promoted ventricular muscle expansion independent of oxygen delivery in an Ino80-dependent manner. Mechanistically, loss of Ino80 resulted in increased E2F-mediated gene expression and S-phase progression. These data support a model where Ino80-mediated control of cell cycle genes is required for coronary angiogenesis. Furthermore, providing two blood vessels sources accelerates myocardial expansion, detexrmining heart wall size and suppressing disease phenotypes.

13. Modulation of Cardiomyocyte Physiology by Stretch-induced miRNAs

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The pathological decline in cardiac function observed during heart failure (HF) correlates with the inability to match contraction with workload at the cellular level. Cardiomyocyte contractility is the result of a coordinated integration of sarcolemma depolarization, Ca2+ handling, energy production and the structural contractility network. Acute pathological stimuli can induce alterations in these processes, making the cardiomyocyte susceptible to contractile dysfunction and arrhythmias, often culminating in HF. However, the mechanisms connecting acute stimuli to the early maladaptive signaling that dictates disease progression remain poorly understood. miRNAs are frequently dysregulated in HF, suggesting a potential role in early remodeling processes. To explore this possibility, we have focused on microRNA control of contractility in the context of hemodynamic stress. We developed a comprehensive approach to deconstruct the interactions between the miRNA landscape and their potential targets during cyclic mechanical stretch. This pipeline has revealed a unique miRNA signature associated with acute stretch, confirmed their individual ability to affect contractile velocity, sarcomere integrity and metabolic function in cardiomyocytes, and identified direct biophysical mRNA targets through AgoIP RISC-Seq analysis. Our approach focusing on acute mechanical stimuli explores previously overlooked target space that may be of therapeutic relevance for early intervention strategies.

14. Imaging Transparent Intact Cardiac Tissue with Single-Cell Resolution

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Introduction: Tissue clearing methods, together with immunofluorescence and modern imaging techniques, have broken the limits of traditional research, upgrading histological study from a 2D to a 3D scale. Due to the distinct structures of different organs, the clearing conditions differ. Most of the clearing agents were designed for brain tissues, the specific clearing conditions and agents for cardiac tissues have never been studied up until now, and 3D microstructures of intact heart tissues as well as fibroblasts phenotype changes after myocardial infarction remodeling have not been displayed at an integral level. Methods & Materials: We used mouse cardiac sections to look for a specific cardiac tissue clearing condition, and circulation perfusion was tested for whole-heart clearing. Immunofluorescence and 3D reconstruction were used to locate the relative space distribution of different molecules in cardiac tissues and to show dynamically the fibroblasts phenotype changing after myocardial infarction in a stereoscopic pattern. For immunofluorescence, the role of electric force was explored to achieve fast antibody labeling. Conclusions: A new convenient and effective cardiac tissue clearing agent SUT (Scheme Update on tissue Transparency) was developed, and whole-heart clearing of mouse, rat and pig were achieved by circulation perfusion. The relative 3D space distribution of different molecules were shown, while the procession of fibroblast phenotype changes were displayed at an integral level with single-cell resolution. EAL (Electrophoretic Antibody Labeling) was created for fast antibody labeling to acquire high-efficiency immunofluorescence.

15. The Contraction and Maturity of Cardiomyocytes Derived from Pluripotent Stem Cells is Influenced by Scaffold Microtopography

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Over 15 million people suffer from coronary heart disease (CHD), which is characterized by narrowing of the coronary arteries that supply blood flow to the heart, which may ultimately lead to heart failure. Current cell-based clinical trials to restore cardiomyocyte (CM) health by local delivery of cells have shown only moderate benefit in improving cardiac pumping capacity. Engineering of myocardial tissue constructs is a promising approach for treatment of coronary heart disease. To engineer myocardial tissues that better mimic the highly ordered physiological arrangement and function of native cardiomyocytes, we generated electrospun microfibrous polycaprolactone scaffolds with either randomly oriented or parallel-aligned microfiber arrangement. Additionally, the scaffolds were co-seeded with human induced pluripotent stem cell-derived cardiomyocytes (iCMs) and endothelial cells (iECs) for up to 12 days after iCM seeding. We demonstrated that aligned microfibrous scaffolds induced iCM alignment along the direction of the aligned microfibers and promoted greater iCM maturation by increasing the sarcomeric length and gene expression of myosin heavy chain adult isoform (MYH7), in comparison to randomly oriented scaffolds. Anisotropy also promoted significantly higher maximum contraction velocity of iCMs cultured after 12 days, compared to randomly oriented scaffolds. Co-seeding of iCMs with iECs on scaffolds led to reduced contractility, compared to when iCMs were seeded alone. These findings demonstrate a dominant role of scaffold anisotropy in engineering cardiovascular tissues that maintain iCM organization and contractile function.

16. Studying Cardiovascular Effects of Marijuana on Healthy Individuals Using Human-derived Induced Pluripotent Stem Cells

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Marijuana is the most widely used illicit drug worldwide. In the US, eight states have legalized recreational marijuana use to date, including California. The recreational use of marijuana is also expanding rapidly in Asia and Europe. However, compared to other widely used drugs such as alcohol or cigarettes, less is known about its adverse effects. Epidemiological studies indicate that marijuana smoking increased the risk of coronary artery disease (CAD). Adverse cardiovascular, cerebrovascular, and peripheral vascular effects also have been reported to be associated with marijuana use. These studies suggest that marijuana use may impair vascular endothelial function. Here we investigate the effect of Δ9-tetrahydrocannabinol (Δ9-THC) on vasculature using human induced pluripotent stem cell-endothelial cells (iPSC-ECs). Δ9-THC induced cytotoxicity in iPSC-ECs and human endothelial cells, without showing apparent cytotoxicity towards cardiac fibroblasts and cardiomyocytes. The mRNA expression of inflammation, oxidative stress, angiogenesis, and migration-related genes were induced in iPSC-ECs after Δ9-THC treatment. Δ9-THC-induced inflammation persisted 8-10 days. These results suggest that Δ9-THC causes endothelial activation in iPSC-ECs. In addition, endothelial dysfunction was found in Δ9-THC-treated iPSC-ECs, with induced inflammation, increased oxidative stress, and impaired angiogenesis and migration abilities. We found genistein, a flavonoid abundantly present in soybeans, could

attenuate Δ9-THC-induced endothelial activation and dysfunction in iPSC-ECs. Taken together, these results indicate that marijuana use cause endothelial activation and dysfunction in healthy individuals, thereby increasing the risk for CAD and vascular diseases. Genistein shows potential in the prevention of marijuana-impaired vascular endothelial function.

17. Restore Impaired Diastolic Function in Induced Pluripotent Stem Cell Derived Cardiomyocytes From Hypertrophic Cardiomyopathy Patients

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Diastolic dysfunction (DD) is commonly seen in hypertrophic cardiomyopathy (HCM), yet the cellular mechanism of DD is not fully understood, and no specific treatment so far has been developed. Patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) hold the potential for disease modeling, mechanism study and drug screening of DD in HCM. In the current study, beating iPSC-CMs were generated from healthy and DD patients carrying familial HCM mutations (MYH7 R663H, TPM1 N835H, and MYBPC3 V321M). Sarcomere shortening measurement with micropatterned iPSC-CMs showed impaired diastolic function as prolonged relaxation time and reduced relaxation rate in HCM cells. Fura-2 AM calcium imaging indicated elevated diastolic calcium and abnormal calcium handlings in HCM iPSC-CMs, which were exaggerated by the β-adrenergic challenge. Combining calcium handling and traction force microscopy (TFM), we found the index of calcium sensitivity (measured as dF/Δ[Ca2+]i) was enhanced by HCM mutations. Thus, both diastolic calcium overload and myofilament sensitization lead to impaired relaxation of HCM cells. To retain diastolic function in HCM iPSC-CMs, we restored calcium homeostasis in diseased cells by partially blockade of the calcium or late sodium current. Our results show the treatments have reset the diastolic calcium level, restored the diastolic function, restricted CaMKII activity and improved long-term survival of HCM iPSC-CMs, suggesting calcium signaling remodeling as an important intracellular pathological mechanism of DD. In summary, the current study developed HCM patient specific iPSC-CM models that recapitulated DD at single cell level, and revealed novel cellular mechanisms and potential therapeutic targets of DD in HCM cardiomyocytes.

18. Topical Application of IcyHot Reduces Myocardial Infarct Size in Rodents by a TRPA1-dependent Mechanism

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Background: Toxic reactive aldehydes are formed during ischemia-reperfusion. The ion channel transient receptor potential ankryin1 (TRPA1) is irreversibly modified by reactive aldehydes which can cause calcium influx and cell death. Here we tested whether topically applied creams containing a reversible TRPA1 agonist could reduce ishchemia-reperfusion injury. Methods: Male Sprague-Dawley rats 8-10 weeks age were subjected to an in vivo myocardial ischemia-reperfusion model of 30 minutes of left anterior descending (LAD) coronary artery ischemia followed by 2 hours reperfusion. Prior to ischemia, rats were untreated or had 1g of cream applied to the abdomen. The creams tested were IcyHot, Bengay, Tiger Balm, or Preparation H. The heart were negatively stained for the area at risk and the infarct size was determined by using TTC staining. A subset of rodents also received an intravenous bolus of the TRPA1 antagonist TCS-5861528 (1mg/kg) or AP-18 (1mg/kg) prior to receiving IcyHot. Further, we isolated adult rat cardiac myocytes and subjected the cardiac myocytes to hypoxia-reoxygenation to evaluate the effect of methyl salicylate (an active ingredient of IcyHot).
Results: Interestingly, both IcyHot and Bengay reduced myocardial infarct size compared to untreated rodents

(IcyHot: 41±3%*, Bengay: 50±2%*, vs control: 62±1%, infarct size/area at risk%, n=6/group, *P<0.05). Giving a TRPA1 antagonist prior to IcyHot blocked the reduction in infarct size. In isolated cardiac myocytes, methyl salicylate (MS), an ingredient of IcyHot, reduced cardiac myocyte cell death by percentage of lactate dehydrogenase (LDH) release when given during reoxygenation (MS 1mM: 33±5%*, MS 2mM: 26±7%* vs DMSO: 59±9%, n=9/group, *P<0.05). The plasma concentration of salicylate, which is metabolite of MS in IcyHot increased during the reperfusion. Conclusion: Since IcyHot and Bengay are safely used by humans, products such as these could be quickly translatable and widely used to reduce ischemia-reperfusion injury.

19. The Role of Collagen Nanofibrillar Alignment in Endothelial Cell Function and Survival

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Research Background & Objectives: Endothelial cells (ECs) are a promising cell type for the treatment of peripheral arterial disease (PAD). Our lab previously showed that anisotropic nanofibrillar scaffolds augment the angiogenic function of human ECs. In order to optimize the biomechanical properties of nanofibrillar scaffolds, we examined the effect of nanofibril size and crosslinking on endothelial survival and function. Methodology: Aligned nanofibrillar collagen scaffolds were fabricated using a shear-based method. Scaffold formulations of varying fibril diameters (90-160 nm) were prepared by altering the ionic strength of monomeric collagen. Scaffolds were further crosslinked at varying levels, and characterized for surface topography and degradation rate. Human ECs were seeded onto the scaffolds for assessment of migration and survival under hypoxia. Results: Scaffold formulation with low ionic strength presents low diameter (LD) fibrils (~90 nm), compared to the high ionic strength formulation group (~160 nm). HD scaffold possesses higher elastic modulus than LD group (20.74 ± 11.73 mPa vs. 13.23 ± 6.17 mPa). The mean direction of migration with respect to the fibril axis was 37.06° on HD, compared to 48.45° on LD, regardless of the degree of crosslinking. Cell survival was ~11% higher on HD scaffolds than that on LD group after culture in 1% O2 for 3 days to mimic tissue ischemia. These data suggest that aligned HD scaffolds promote EC survival and alignment along the fibril direction. Significance: This study provides new insight into the role of biophysical microenvironment in the survival, migration and angiogenesis of ECs. The findings may lead to a novel clinical strategy for the therapeutic neovascularization of PAD patients.

20. Novel In Vivo miRNA Delivery with Nanoparticles by Injectable Hydrogel for Cardiovascular Function Improvement

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Cardiovascular disease (CVD) is the No. 1 killer in the United States, and one of leading causes is heart failure caused by myocardial infarction (MI). A number of new approaches have been reported to restore the worsening condition of infarcted myocardium via either exogenous stem cell transplantation, or growth factor and miRNA based interventions to stimulate the intrinsic rejuvenating mechanisms. In this study, we have established a novel in vivo delivery system with nanoparticles (NPs) as miRNA carriers and elastin-based injectable hydrogel as vehicle to transport the miRNA into the infarcted myocardium to achieve cardiovascular function improvement. First, the NPs with core-shell structure at ~100 nm in diameter has been successfully synthesized to encapsulate the miRNA with intact length and function. Then, the uptake efficiency of NP in human

embryonic stem cell -cardiomyocyte (hESC-CMs) and -endothelial cells (hESC-ECs) were higher than 60% at varied loading concentrations showing less toxicity than that of the lipofectamine RNAiMAX transfection. The in vivo rat study with NP/hydrogel injections demonstrated the improved cardiac function with increased ejection fraction (EF, echo) by 20%, smaller scar size by 10%, and higher capillary density in the scar area in 4 weeks. Further, the hESC-CM and hESC-EC in vitro modeling were utilized to confirm the cellular and molecular mechanisms on the cardiovascular function improvement. In summary, the novelty of this in vivo miRNA delivery system via NPs and injectable hydrogel stands on the high transfection efficiency and cell viability of cardiovascular cells and fast cardiac contraction adaption of the hydrogel in a very convenient therapeutic approach.

21. Myocardial Reparative Functions of Exosomes From MSCs are Enhanced by Hypoxia Treatment of the Cells via Fransferring microRNA-210

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Hypoxia treatment enhances paracrine effect of mesenchymal stem cells (MSCs). The aim of this study was to investigate whether exosomes from hypoxia-treated MSCs (ExoH) are superior to those from normoxia-treated MSCs (ExoN) for myocardial repair. Mouse bone marrow-derived MSCs were cultured under hypoxia or normoxia for 24 hours, and exosomes from conditioned media were intramyocardially injected into the infarcted heart of C57BL/6 mice. ExoH resulted in significantly higher survival, smaller scar size and better cardiac functions recovery. ExoH conferred increased vascular density, lower cardiomyocyte apoptosis, reduced fibrosis, and increased recruitment of cardiac progenitor cells in the infarcted heart relative to ExoN. MicroRNA analysis revealed significantly higher levels of microRNA-210 (miR-210) in ExoH compared with ExoN. Transfection of a miR-210 mimic into ECs and CMs conferred similar biological effects as ExoH. Hypoxia treatment of MSCs increased the expression of neutral sphingomyelinase 2 (nSMase2) which is crucial for exosome secretion. Blocking the activity of nSMase2 resulted in reduced miR-210 secretion and abrogated the beneficial effects of ExoH. In conclusion, hypoxic culture augments miR-210 and nSMase2 activities in MSCs and their secreted exosomes, and this is responsible at least in part for the enhanced cardioprotective actions of exosomes derived from hypoxia-treated cells.

22. Modified mRNA Transcript-incorporated Alginate Scaffold as Controlled Delivery System for Angiogenic Factors mRNA, for Revascularization of Ischemic Skeletal Muscles

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Massive muscle injuries caused by traumatic or surgical actions may significantly impair the regeneration and revascularization capability of skeletal muscle. As an alternative to reconstructive surgery, biomimetic scaffolds capable of providing an efficient platform for endogenous muscle regeneration and potentially inducing revascularization through therapeutic angiogenesis have received great interest during last few years. The recent advances in enhancing the stability of mRNA through chemical modifications have introduced modified mRNA (mmRNA) as a potential therapeutic candidate for various applications including angiogenesis. Modified mRNA delivery may represent a safer and more promising strategy of therapeutic angiogenesis compared to gene and protein delivery, by inducing the expression of angiogenic proteins through the cells' translational machinery, without integrating into the genome. The aim of this study was to develop mmRNA transcript-incorporated scaffolds as a controlled delivery system for angiogenic factors, for revascularization of ischemic skeletal muscles. The effect of physicochemical properties of the porous alginate scaffold on mmRNA release kinetics, transfection efficiency, and cell viability was studied. Taking advantage of electrostatic interactions

between the negatively charged alginate scaffold and cationic lipid/mmRNA complexes, sustained release of GFP mmRNA was obtained for up to one week. The live/dead cell viability studies demonstrated high cell viability of human microvascular endothelial cells (HMEC-1) on alginate scaffold at a lower degree of cross-linking. The mmRNA transcript-incorporated scaffold can be potentially employed as a promising therapeutic approach for revascularization in broad range of ischemic diseases.

23. TBX5-Clover2/NKX2-5-TagRFP iPSCs for Simultaneously Isolating Human Lineage-Specific Cardiovascular Cells

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Heterogeneity and immaturity of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) significantly impede their applications in regenerative medicine, disease modelling, and personalized drug testing. Here, we developed a method for simultaneous isolation of lineage-specific ventricular-like, atrial-like and nodal-like CMs with high purity using a TBX5-Clover2/NKX2-5-TagRFP hiPSC reporter system. We also demonstrate that TBX5+/NKX2-5+ CMs possess more mature electrophysiological and metabolic phenotypes.

24. High Spatiotemporal Resolution Mapping of Cardiac Arrhythmic Activities in Porcine Model Using a Novel Elastic Polymeric Device

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Atrial fibrillation (AF) is the most prevalent arrhythmia affecting 3-6 million U.S. individuals, and is a major contributor to morbidity and mortality. Recently, a fully stretchable microscale sensor array has been developed, which provides intimate coupling and enables us to map cardiac arrhythmia activities with high spatiotemporal resolution. We have successfully demonstrated that the electrode array is capable of measuring epicardial cardiac arrhythmic activities, corresponding to simultaneous endocardial basket catheter mapping in an in vivo porcine model. This will enable us to better track and understand initiation and maintenance of atrial fibrillation.

25. Cell Type-Specific Chromatin Signatures Underline Regulatory DNA Elements in Human Induced Pluripotent Stem Cells and Somatic Cells

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Rationale: Regulatory DNA elements in the human genome play important roles in determining the transcriptional abundance and spatiotemporal gene expression during embryonic heart development and somatic cell reprogramming. It is a mystery how chromatin marks in regulatory DNA elements are modulated to establish

cell type-specific gene expression in the human heart. Objective: We aimed to decipher the cell type-specific epigenetic signatures in regulatory DNA elements and how they modulate heart-specific gene expression. Methods and Results: We profiled genome-wide transcriptional activity and a variety of epigenetic marks in the regulatory DNA elements using massive RNA-seq (n=12) and ChIP-seq (n=84) in human endothelial cells (ECs: CD31+CD144+), cardiac progenitor cells (CPCs: Sca1+), fibroblasts (FBs: DDR2+), and their respective induced pluripotent stem cells (iPSCs). We uncovered two classes of regulatory DNA elements: Class I was identified with ubiquitous enhancer (H3K4me1) and promoter (H3K4me3) marks in all cell types, whereas Class II was enriched with H3K4me1 and H3K4me3 in a cell type-specific manner. Both Class I and Class II regulatory elements exhibited stimulatory roles in nearby gene expression in a given cell type. However, Class I promoters displayed more dominant regulatory effects on transcriptional abundance regardless of distal enhancers. Transcription factor network analysis indicated that human iPSCs and somatic cells from the heart selected their preferential regulatory elements to maintain cell type-specific gene expression. In addition, we validated the function of these enhancer elements in transgenic mouse embryos and human cells, and identified a few enhancers that could possibly regulate the cardiac-specific gene expression. Conclusions: Given that a large number of genetic variants associated with human diseases are located in regulatory DNA elements, our study provides valuable resources for deciphering the epigenetic modulation of regulatory DNA elements that fine-tune spatiotemporal gene expression in human cardiac development and diseases.

26. A Shape-controlled Tunable Microgel Cell Delivery Platform for Low-dose Delivery of Primed Stem Cells for In Vivo Therapeutic Neovascularization

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Hydrogel-based cell delivery platforms fabricated from biomaterials have evolved from a cytoprotective design to a functional biomaterial design. During natural tissue regeneration, cellular microenvironment and soluble cues influence reparative mesenchymal stem cells (MSCs). Prolonged ischemia and lack of blood supply drive cellular apoptosis and tissue death. However, delivery of primed stem cells via a tunable microenvironment triggers an 'angiocrine' response, driving therapeutic angiogenesis1. In this study, it is hypothesised that delivery of primed human MSCs on a shape-controlled microgel platform at a low-cell dose promotes angiogenesis in a hindlimb ischemia (HLI) model. Optimized 2mg/ml collagen microgels were fabricated by dispensing type-I collagen with 4S-Star-PEG and hMSCs at 0.8x106 cell density on to a hydrophobic surface1. Balb/c nude mice underwent unilateral HLI and were divided into five groups (n=12/group); PBS; microgels alone; microgels with 50,000 hMSCs; 50,000 and 1,000,000 cells alone. Laser-Doppler perfusion and pathological markers of severity were assessed between the groups. Histological and molecular evaluation of inflammation and angiogenesis were assessed using immunohistochemistry, multiplex ELISA, gene expression arrays and and MALDI-imaging mass spectrometry. Statistical analysis was performed using one-way ANOVA with $p<0.05$. Perfusion analysis revealed higher perfusion in hMSC embedded microgels 60%±20% compared to controls. Immunohistochemistry revealed increased angiogenesis and reduction in inflammation at day 21. hMSC embedded microgels showed significant up-regulation of protein and gene markers related to angiogenesis, and changes in tissue N-glycan profile. Microgel delivery of primed hMSCs at a low-cell dose was demonstrated to promote functional angiogenesis in a severe murine model of HLI. Acknowledgement: Science Foundation Ireland (SFI) under grant no.09/SRC/B1794. Reference: 1. Thomas D. et al, Biomaterials, 35, 8757-66, 2014.


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