

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

		3. DATE RECEIVED BY STATE	State Application Identifier
1. TYPE OF SUBMISSION*		4.a. Federal Identifier HL134283	
<input type="radio"/> Pre-application <input checked="" type="radio"/> Application <input type="radio"/> Changed/Corrected Application		b. Agency Routing Number	
2. DATE SUBMITTED 2016-12-08	Application Identifier PD15-06597	c. Previous Grants.gov Tracking Number	
5. APPLICANT INFORMATION			Organizational DUNS*: 0788615980000
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6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*		1136171197A1	
7. TYPE OF APPLICANT*		O: Private Institution of Higher Education	
Other (Specify):			
Small Business Organization Type		<input type="radio"/> Women Owned	<input type="radio"/> Socially and Economically Disadvantaged
8. TYPE OF APPLICATION*		If Revision, mark appropriate box(es).	
<input type="radio"/> New	<input checked="" type="radio"/> Resubmission	<input type="radio"/> A. Increase Award	<input type="radio"/> B. Decrease Award
<input type="radio"/> Renewal	<input type="radio"/> Continuation	<input type="radio"/> C. Increase Duration	<input type="radio"/> D. Decrease Duration
	<input type="radio"/> Revision	<input type="radio"/> E. Other (specify):	
Is this application being submitted to other agencies?*		<input type="radio"/> Yes	<input checked="" type="radio"/> No
What other Agencies?			
9. NAME OF FEDERAL AGENCY* National Institutes of Health		10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER TITLE:	
11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT* The Role of Exosomes in Mesenchymal Stem Cell-Mediated Enhancement of Cardiac Contractility			
12. PROPOSED PROJECT Start Date* 07/01/2017		13. CONGRESSIONAL DISTRICTS OF APPLICANT Ending Date* 06/30/2021	

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE**Page 2****14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

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15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	\$366,904.00
b. Total Non-Federal Funds*	\$0.00
c. Total Federal & Non-Federal Funds*	\$366,904.00
d. Estimated Program Income*	\$0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

- a. YES THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
 DATE:
- b. NO PROGRAM IS NOT COVERED BY E.O. 12372; OR
 PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

I agree*

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION

File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: Michael Middle Name: J. Last Name*: King Suffix:
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Signature of Authorized Representative*

Mr. Michael J. King

Date Signed*

12/08/2016

20. PRE-APPLICATION File Name:**21. COVER LETTER ATTACHMENT** File Name:Cover Letter Final_JM.pdf

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Project/Performance Site Location(s)

Project/Performance Site Primary Location

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Icahn School of Medicine at Mount Sinai
Duns Number: 0788615980000
Street1*: One Gustave L. Levy Place
Street2:
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 10029-6574
Project/Performance Site Congressional District*: NY-013

Additional Location(s)

File Name:

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* Yes No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? Yes No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6

If NO, is the IRB review Pending? Yes No

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* Yes No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? Yes No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* Yes No**4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*** Yes No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an Yes No environmental assessment (EA) or environmental impact statement (EIS) been performed?

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* Yes No

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* Yes No

6.a. If yes, identify countries:

6.b. Optional Explanation:

Filename

7. Project Summary/Abstract* Project Summary Final.pdf**8. Project Narrative*** Project Narrative Final_1.pdf**9. Bibliography & References Cited** References.pdf**10. Facilities & Other Resources** Facilities and Other Resources Final.pdf**11. Equipment** Major Equipment Final.pdf

PROJECT SUMMARY

An emerging therapy for non-ischemic cardiomyopathy involves the delivery of human mesenchymal stem cells (hMSCs). Clinical trials document modest benefits on cardiac contractility, underscoring a need to better understand and exploit the underlying mechanisms governing hMSCs-cardiomyocyte (hCMs) interactome.

Recent studies on hMSC-mediated heart therapies demonstrated that paracrine signaling—via secreted factors—is a crucial mediator of reduced cardiac fibrosis and enhanced angiogenesis. Moreover, hMSC paracrine factors have been shown to impact contractility by altering cardiomyocyte ion channel/pump activity. However, these findings fail to identify the key components of the hMSC secretome for enhancing contractility. We propose utilizing three-dimensional human engineered cardiac tissues (hECTs) as an *in vitro* model to investigate the role of hMSC exosomes in enhancement of cardiac contractility.

Our lab recently discovered that hMSCs enhance hECT contractile force predominantly through paracrine signaling, counteracting adverse risks of hMSC-hCM heterocellular coupling. Importantly, we discovered that the exosomal component of the hMSC secretome is necessary and sufficient for hMSC-paracrine mediated enhancement of hECT contractility. Furthermore, by utilizing a systems biology approach and integrating hECT contractile function results with exosomal miRNA data, we predicted exosomal miRNA-21 as a lead candidate responsible for the favorable contractile effects of hMSC paracrine signaling. We later validated with qPCR that miRNA-21 levels are increased in hECTs supplemented with hMSC exosomes and hMSC total conditioned media relative to control, motivating our central hypothesis that *exosomal miRNA-21 plays a key role in hMSC paracrine-mediated enhancement of human engineered cardiac tissue contractile performance*.

In testing this hypothesis, I will directly address an NHLBI topic of special interest (HL-142) by studying the role of exosomes as paracrine mediators in cardiovascular disease. In **Aim 1**, I will identify the role of exosomal miRNA-21 in hMSC-mediated enhancement of hECT contractility by: 1) treating hECTs with exosomes derived from hMSCs with miRNA-21 inhibitor (Sub-aim 1A); and 2) formulated lipidoid nanoparticle delivery of miRNA-21 mimic into hECTs (Sub-aim 1B). In **Aim 2**, I will test the role of hMSC exosomes on recovery of contractility using *in vitro* hECT models of acquired (Sub-aim 2A) and genetic (Sub-aim 2B) non-ischemic heart failure.

Overall, the project is designed to frame the research within a clinical context, and provide a rigorous multi-disciplinary training in tissue engineering, systems biology, electrophysiology, stem cell biology, and biochemistry as a solid foundation on which to build my career as a future physician-scientist.

PROJECT NARRATIVE

Clinical trials have shown that transplanting mesenchymal stem cells to non-ischemic cardiomyopathy patients modestly improves cardiac functional capacity, underscoring the need to better understand and exploit the cardiomyocyte-mesenchymal stem cell interactome. This proposal aims to utilize engineered cardiac tissues to study the role of exosomes in mesenchymal stem cell paracrine-mediated enhancement of cardiac contractility. Our study will provide novel insight into the role of exosomes and their cargo as paracrine signaling mediators in cardiovascular disease, and may ultimately help guide future alternative cardiotherapies.

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FACILITIES AND OTHER RESOURCES

Laboratory: The mentor's laboratory facilities constitute one of the main laboratories in the Cardiovascular Research Center (CVRC) of The Icahn School of Medicine at Mount Sinai. Founded in 2007, the CVRC, under the directorship of co-mentor, Dr. Roger Hajjar, includes multiple investigators who are extramurally funded and have various cardiovascular projects focused on biomedical engineering, nanomedicine, developmental biology, electrophysiology/optical mapping, vascular biology, pharmacogenomics, stem cell biology, and gene therapy vector biology. Investigators from these various disciplines join their talents to pursue cross-disciplinary goals using fundamental discoveries to prevent and reverse heart failure and atherosclerosis. Facilities, resources, and knowledge are regularly shared amongst the laboratories. This provides a wealth of expertise for the Applicant to help ensure success of the proposed experiments including stem cell differentiation, and molecular and biochemical assays.

The Costa Laboratory consists of approximately 1000 square feet on the 3rd floor of the Atran Building at Mount Sinai, and is dedicated to Cardiovascular Cell and Tissue Engineering research. The space includes a small machining and electronics shop area for developing custom tissue testing devices and bioreactors for culturing cells and tissues under dynamic, multiaxial loading conditions mimicking the physiologic environment. There is a commercial physiologic tissue bath system (SI Heidelberg) and a custom twitch force monitoring station for testing active and passive mechanical properties of natural and engineered tissue samples, including human engineered cardiac tissues. The laboratory is also equipped with an isolated working heart setup for measuring engineered cardiac chamber pump function, an isolated perfusion system for adult rat heart cardiomyocyte isolation, an Ionoptix 6-well Myopace system for electrically stimulating cardiac cells and tissues during culture, a fabrication area for custom PDMS soft lithography and elastomer development and characterization, a McIlwain tissue chopper for cutting fresh or fixed tissue, a dissecting microscope equipped with high-speed, high-resolution digital camera for imaging beating cardiac tissues, and two sterile clean benches for maintaining sterility while performing tissue function tests. A small animal surgery station includes isoflurane flow regulator (Surgivet), rodent ventilator (Harvard Apparatus), dissecting microscope (Olympus) with camera (PixelLink), warming pad, and glass bead sterilizer (FST). There are several high-end Macintosh and PC personal computers for data analysis, computational modeling, image analysis, and computer-aided design. There is an EVOSfl digital epifluorescent microscope for monitoring cell function, transfection, and immunocytochemistry. Live cell time-lapse imaging is provided by a VivaView FL Incubator Fluorescence Microscope from Olympus, which includes an integrated multi-gas incubator for hypoxia cultures. Cell and tissue culture facilities include 2 culture hoods (Biosafety Level 2), 3 incubators, freezers, centrifuges, autoclave, shaker bath, shared cold room, and other equipment for primary cell isolation and tissue culture. Human stem cell culture and differentiation is performed in an adjacent 150 square foot fully equipped shared cell culture room that includes one multi-gas incubator for hypoxic cell culture.

The Hajjar Laboratory. The hub of the Cardiovascular Research Center (CVRC), the Hajjar Laboratory consists of approximately 2500 square feet on the 7th floor of the new Leon and Norma Hess Center for Science and Medicine, located near the Atran Building with direct access by underground tunnels. The Hajjar Lab is fully equipped with facilities, equipment, and resources necessary for performing studies in molecular biology, differentiated and stem cell biology, viral vector development and delivery, optical microscopy, and in-vivo biology. For such purposes, equipment readily available in the Hajjar lab includes 3 Eppendorf thermocyclers, 1 Eppendorf real-time PCR machine, six Western blot systems, an X-ray film developer for autoradiograph development, 2 Leica cryostats, and one Leica microtome for tissue sectioning. All equipment and reagents necessary for tissue staining and immunohistochemistry are readily available. Two epifluorescent microscopes are available for immunocytochemistry. Cell and tissue culture facilities, located adjacent to the lab in three dedicated rooms, include 9 culture hoods (Biosafety Level 2), 17 incubators, multiple refrigerators, -20°C and -80°C freezers and a walk in cold room. One of the three rooms is dedicated solely to the culture and differentiation of embryonic stem cells, and includes 2 hoods and three gas controlled incubators. Additionally, the lab contains multiple low, medium, and high-speed centrifuges, as well as a Beckman XE100Ultra ultracentrifuge. The lab space also includes multiple autoclaves, several shaker baths, and other equipment for primary cell isolation and tissue culture.

Computer: The Costa lab houses multiple Macintosh and PC personal computers and workstations for data acquisition and analysis, computational modeling, image processing, and computer aided design. There is also a flatbed scanner, and color laser printer. Each postdoctoral fellow and graduate student has his/her own

workstation to conduct data analysis and research. The computer network is connected to the Mount Sinai Ethernet, which provides full Internet access.

Office: Dr. Costa's office (Atran Rm 3-39) is approximately 100 square feet that includes a personal computer, telephone with conference call and speaker options, multifunction printer/scanner/copier, and small meeting table. This office opens directly into the Costa laboratory.

CORE FACILITIES:

Flow Cytometry Shared Resource Facility: Located in the Icahn Medical Institute (IMI) building across Madison Avenue from the mentor's laboratory, the Flow Cytometry SRF provides instrumentation and expertise for automated cytofluorimetric analysis and the sterile sorting of specific cell types. The equipment of this facility is state-of-the-art; it consists of three high-speed cell sorters: MoFlo (Dako-Cytomation), Vantage (Becton & Dickinson) and Influx (Cytopeia-BD) equipped with three lasers and a single deposition unit; three analytical flow cytometers: FACScan and FACScalibur with CellQuest software and a state-of-the-art 5 laser-LSR II with Diva software (all from BD), for up to 12-color analysis. The LSR II is also equipped with High Throughput Sampler, which allows high speed and automated sample acquisition from microtiter plates. The IMI and Atran Buildings are directly connected by an underground passageway.

Microscopy Shared Resource Facility: Located in the nearby Annenberg Building, the Microscopy SRF provides equipment, instruction, and expertise for the microscopic examination of cells and tissues. It has capabilities for brightfield and fluorescence microscopy, including the Zeiss AxioPlan2, an upright epifluorescence microscope controlled by Zeiss AxioVision software. This microscope has a Zeiss AxioCam MRm camera for capturing fluorescent dyes and a Q-imaging MP3.3 RTV color camera for capturing full color brightfield images; it also has an ApoTome unit that uses "structured illumination" to perform real-time deconvolution of tissues and cells. Furthermore, it can perform transmitted light imaging techniques, such as darkfield, polarized light, and DIC. The facility also has instruments for confocal, multiphoton, live cell, and electron microscopy, as well as a number of software packages for image analysis including Metamorph, AutoDeblur, Amira, and Volocity. In particular, the Leica TCS-SP is mated to an inverted microscope with a new Pentium 4 computer and powerful Leica LCS software; this system will excite fluorophores from UV to far red using an Ar-UV laser (350nm), an Argon laser (488nm), a Diode laser (561nm), and a HeNe laser (633nm). The spectrophotometer scan head allows the user to "tune" the detectors to any emission wavelength. The microscope can scan up to 4 channels simultaneously plus a transmitted light channel and will perform spectral separation of overlapping fluorophores.

Human Embryonic Stem Cell (hESC) Core: Located in the IMI building, the hESC core provides undifferentiated hESCs, mouse embryonic fibroblasts and/or hematopoietic, cardiac and endoderm progenitors; it also provides training to faculty, postdoctoral fellows, and students in the maintenance and the differentiation of hESCs. Material and reagents necessary for maintenance, as well as differentiation of hESCs, are also available. A part of the core is also dedicated to the development of novel induced pluripotent stem cells (iPS) cell lines and their differentiation, to assist investigators with the development of iPS cell lines from human patients. Troubleshooting and consulting support for human mesenchymal stem cell (hMSC) research is also available.

Genomics Core: We will use the Mount Sinai Genomics Core facility at the 13th floor, Icahn Medical Institute building, which is located across the street from Hess Building, Mount Sinai. The genomics core is a CLIA-certified laboratory, which houses next-generation sequencing capabilities for basic research and clinical applications. The Genomics Core is directed by Dr. Milind Mahajan, who has access to a number of technology platforms available for use including Nucleic Acid characterization and bioinformatics support. The genomics core has well-established Illumina sequencing pipelines and next-generation sample preparation will be achieved using TruSeq RNA Library Preparation kit (v2) followed by massively parallel sequencing with the sequencing machines such as Illumina HiSeq 2500 system. These kits and equipment will be used for our proposal relevant to miRNA profiling in stem cell exosomes. Our samples will be multiplexed using unique barcode indexing system and run in a single illumina flowcell lane. The genomics core also has Agilent bioanalyzer facility, which we will use to ensure the quality of our exosomal RNA samples. With a strongly established track record, the genomics core at Mount Sinai will be an ideal facility for the proposed

experimental approaches involving next-generation sequencing of RNA. To further validate datasets generated by next-generation sequencing, we will use High Capacity cDNA synthesis kit and or miRNA Taqman assays to synthesize cDNA from representative samples to validate miRNA candidates identified by NGS and bioinformatics. The Sahoo laboratory at the CVRC facility at the Hess building has Mastercycler Pro (eppendorf) and Fast Realtime PCR 7500 (applied biosystems) for miRNA validation.

OTHER RESOURCES

Other relevant resources available to the Mount Sinai research community include the renowned Translational and Molecular Imaging Institute, the Black Family Stem Cell Institute, and the Zena and Michael A. Wiener Cardiovascular Institute. There is a fully equipped machine shop for custom fabrication and design, and a new Rapid Prototyping Center for 3D printing, laser cutting, CNC machining, and other facilities for custom electrical and mechanical device design and manufacturing. Outstanding information resources include school wide libraries at Mount Sinai School of Medicine. The Mount Sinai Hospital and School of Medicine campus occupies a highly concentrated area on the upper east side of Manhattan, so that all of these facilities are within close proximity for easy access. Most shared facilities operate on a fee-for-service basis, typically costing from \$25 to \$50 per hour, with costs defrayed by the mentor's research funds.

SCIENTIFIC ENVIRONMENT:

The Icahn School of Medicine at Mount Sinai is a collaborative, supportive, and stimulating MD/PhD training environment. The Cardiovascular Research Center, the scientific hub of Mount Sinai Heart, particularly emphasizes interdisciplinary and translational research, and offers a weekly seminar series, a broad base of expertise, and strong resource support. The CVRC includes clinicians and investigators in biomedical engineering, nanomedicine, developmental biology, electrophysiology/optical mapping, vascular biology, pharmacogenomics, stem cell biology, and gene therapy vector biology. The specifics of the research environment, particularly how the environment supports the professional development of the applicant as well as the proposed work, are discussed in detail in the Sponsor Information portion of the application.

MAJOR EQUIPMENT

Engineered Cardiac Tissue Testing: The Costa Lab is fully equipped and actively functional for the proposed engineered cardiac tissue contractile testing experiments. Key equipment includes two laminar flow hoods (NUAIRE) with a custom mounted stage, that includes a vibration isolation table, a heating stage, a GRASS stimulator for electrical pacing experiments, a dissecting microscope connected to a high speed camera, and a personal computer for live imaging and data acquisition. Using custom LabView software, the system allows real-time tracking of cantilever post deflection as the hECTs are beating. Data post-processing is performed using established algorithms for automated twitch force analysis using custom MatLab code with a user-friendly GUI interface.

Cell and Tissue Culture Facilities: In the Costa Lab, cell and tissue culture facilities (Biosafety Level 2) include 2 incubators, -20°C and -80°C freezers, a walk in cold room, centrifuges, autoclave, shaker baths, and other equipment for primary cell isolation and tissue culture. A designated cell culture room is equipped with 2 sterile hoods as well as 3 standard incubators and 1 multi-gas incubator.

In the Hajjar lab, cell and tissue culture facilities, located adjacent to the lab in three dedicated rooms, include 9 culture hoods (Biosafety Level 2) and 17 incubators. The general lab space includes multiple refrigerators, -20°C and -80°C freezers, and a walk in cold room. One of the three tissue culture rooms is dedicated solely to the culture and differentiation of embryonic stem cells and includes 2 hoods and three gas controlled incubators. Areas are segregated for specific cell culture types to avoid cross contamination between adult cell culture, stem cell culture, and gene delivery program. The lab contains 6 Eppendorf low-speed and 3 Eppendorf 5810R medium speed centrifuges, 2 Sorvall RC 6 Plus floor centrifuges, and a Beckman XE100Ultra ultracentrifuge. The lab space also includes multiple autoclaves, several shaker baths, and other equipment for primary cell isolation and tissue culture.

Imaging: There is an EVOSfl digital epifluorescent microscope and an Olympus VivaView long-term time lapse imaging system. This equipment is located within the Costa Laboratory, and the applicant has immediate access to it. In the Hajjar lab, an Olympus 1x71 and Nikon E400 are available for imaging of immunocytochemistry.

Beckman Ultracentrifuge: Two shared Beckman L8 M and L5-508 Ultracentrifuges are conveniently located in the CVRC near the culture room and cold room for storage of rotors, and will be used for isolation of exosomes.

Dynamic Light Scattering (DLS): Adjacent to her laboratory, Dr. Sahoo, has access to a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) instrument used to analyze exosome samples.

Molecular Biology: The laboratory is fully equipped with tools for molecular biology including: 3 Eppendorf thermocyclers, 1 Eppendorf real-time PCR machine, six Western blot systems, a Protec Medical Systems ECOMAX developer for autoradiograph development, 2 Leica cryostats, and one Leica microtome for tissue sectioning. All equipment and reagents necessary for tissue staining and immunohistochemistry are readily available.

Electron Microscopy: An Hitachi H7650 transmission electron microscope (TEM) and an S-4300 scanning electron microscope (SEM) that are located in the MSSM Pathology Electron Microscopy Core on the 6th floor of the Atran building can be used to image and characterize prepared exosome samples.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: Joshua	Middle Name	Last Name*: Mayourian	Suffix:
Position/Title*:	M.D./Ph.D Student			
Organization Name*:	Icahn School of Medicine at Mount Sinai			
Department:	Medicine - Cardiology			
Division:				
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Street2:				
City*:	New York			
County:	New York			
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	10029-6574			
Phone Number*:	212-824-8904	Fax Number:		
E-Mail*:	joshua.mayourian@icahn.mssm.edu			
Credential, e.g., agency login:	jmayour			
Project Role*:	PD/PI	Other Project Role Category:		
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	JM Biosketch Final_1.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix: Dr.	First Name*: Kevin	Middle Name D.	Last Name*: Costa	Suffix:
Position/Title*:	Associate Professor			
Organization Name*:	Icahn School of Medicine at Mount Sinai			
Department:	Medicine - Cardiology			
Division:				
Street1*:	One Gustave L. Levy Place			
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City*:	New York			
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State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	10029-6574			
Phone Number*:	212-241-7122		Fax Number: 646-537-9458	
E-Mail*:	kevin.costa@mssm.edu			
Credential, e.g., agency login:	KCOSTA			
Project Role*:	Other (Specify)		Other Project Role Category: Sponsor	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	Costa Biosketch Final.pdf		
Attach Current & Pending Support:	File Name:			

PROFILE - Senior/Key Person				
Prefix: Dr.	First Name*: Roger	Middle Name Joseph	Last Name*: Hajjar	Suffix:
Position/Title*:	Director and Professor			
Organization Name*:	Icahn School of Medicine at Mount Sinai			
Department:	Medicine - Cardiology			
Division:				
Street1*:	Icahn School of Medicine at Mount S			
Street2:	Cardiovascular Research Center			
City*:	New York			
County:	New York			
State*:	NY: New York			
Province:				
Country*:	USA: UNITED STATES			
Zip / Postal Code*:	10029-6574			
Phone Number*:	212-824-8904		Fax Number: 212-241-4080	
E-Mail*:	roger.hajjar@mssm.edu			
Credential, e.g., agency login:	RJHAJJAR			
Project Role*:	Other (Specify)		Other Project Role Category: Co-Sponsor	
Degree Type:	Degree Year:			
Attach Biographical Sketch*:	File Name:	RJH Biosketch Final.pdf		
Attach Current & Pending Support:	File Name:			

APPLICANT BIOGRAPHICAL SKETCH

DO NOT EXCEED FIVE PAGES.

NAME OF APPLICANT: Joshua Mayourian

eRA COMMONS USER NAME: jmayour

POSITION TITLE: MD/PhD Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	START DATE (MM/YYYY)	END DATE (MM/YYYY)	FIELD OF STUDY
Cooper Union for the Advancement of Science and Art	Bachelor of Engineering	09/2010	05/2014	Chemical Engineering (Major) Biomedical Engineering (Minor)
Cooper Union for the Advancement of Science and Art	Master of Engineering	09/2013	05/2014	Chemical Engineering (Major)
Icahn School of Medicine at Mount Sinai	MD/PhD Candidate	07/2014	Present	MD/PhD Student in Biomedical Science

A. Personal Statement

My long-term research goal is to utilize cardiac tissue engineering and computational approaches to develop superior stem cell-based cardiac therapies. My undergraduate and graduate academic training at Cooper Union, as well as my cardiac tissue engineering research experiences at the Icahn School of Medicine at Mount Sinai (ISMMS), have provided me with the tools necessary to accomplish this goal. As a rising junior chemical engineering student at Cooper Union, I was accepted into the competitive Summer Undergraduate Research Program at the ISMMS, where I joined Dr. Kevin Costa's laboratory. During that summer, I designed an increased throughput bioreactor for cardiac tissue engineering to isolate heterocellular coupling and paracrine signaling mechanisms, and mathematically modeled paracrine signaling between mesenchymal stem cells and cardiomyocytes. This research led to a podium presentation at the annual Biomedical Engineering Society meeting in Atlanta, Georgia, as well as co-authorship on a related conference abstract. Following this experience, I was accepted early assurance into the MD/PhD program at ISMMS, and was invited back into Dr. Costa's lab for another summer rotation the following year. During the second rotation, I developed the first mathematical model of mesenchymal stem cell electrophysiology, and coupled this model to established human cardiomyocyte electrophysiology models. This research led to a first author manuscript (cover article in *PLoS Comp Bio*), a Master's degree concurrent with my Bachelor's degree in Chemical Engineering, as well as an accepted poster presentation and travel award at the 2016 Annual Biophysical Society meeting in Los Angeles, California. My potential for integrating cardiac tissue engineering and computational modeling was recognized by the IBM Watson Research Center and Mount Sinai; specifically, my research led to an internship at IBM, and a competitive Graduate Fellowship from the Mount Sinai Institute of Technology. My passion for applying bioengineering approaches throughout my academic training and research guided me to stay in Dr. Costa's lab for my doctoral dissertation, where I will continue studying mesenchymal stem cell-mediated cardiac therapies in order further develop a rigorous multi-disciplinary education in tissue engineering, systems biology, stem cell biology, and exosome signaling. Overall, I believe my sponsor, research project, and the training from this fellowship will provide a strong foundation in order for me to achieve my ultimate career goal as an independent physician-scientist in translational cardiology.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Tutor	09/09	Present	Biology, Mathematics, and Physics	Self-employed	Self-employed
Chemistry Researcher	01/12	06/12	Organic Chemistry	Cooper Union	Ruben M. Savitzky, PhD
Summer Undergraduate Research Fellow	06/12	10/12	Cardiac Tissue Engineering	Icahn School of Medicine at Mount Sinai	Kevin D. Costa, PhD
Teaching Assistant	01/13	06/13	Bioelectricity	Cooper Union	Nina Tandon, PhD
Summer Undergraduate Research Fellow	06/13	08/13	Cardiac Electrophysiology	Icahn School of Medicine at Mount Sinai	Kevin D. Costa, PhD
Graduate Teaching Assistant	1/16	Present	Medical Physiology: Cardiovascular Block	Icahn School of Medicine at Mount Sinai	Staci Leisman, MD
IBM Thomas J. Watson Intern	06/16	09/16	Cardiac Electromechanics	IBM Thomas J. Watson	Viatcheslav Gurev, PhD

Academic and Professional Honors

Full-tuition scholarship, Cooper Union, 2010-2014

Dean's list, Cooper Union, 2010-2014

Early Assurance Acceptance into the Icahn School of Medicine at Mount Sinai MD/PhD Program, 2012

Goldwater Scholarship Honorable Mention, 2013

Responsible for Greatness Award, Cooper Union, 2013

Daniel Okrent Cooper Fund Scholar, Cooper Union, 2013

Herbert Baldwin Fund Prize, Cooper Union, 2014

Elmer J. Badin Chemistry Award, Cooper Union 2014

Summa Cum Laude, Cooper Union, 2014

Icahn School of Medicine MSTP training grant (NIH T32-GM007280), 2014-2015

Rudin Fellow, Icahn School of Medicine at Mount Sinai, 2014-2015

Mount Sinai Institute of Technology Fellow, Icahn School of Medicine at Mount Sinai, 2015

Travel Award, Biophysical Society, 2016

Integrated Pharmacological Sciences Training Program (NIH T32-GM062754), NIGMS, 2016

IBM Thomas J. Watson Internship, IBM Thomas J. Watson, 2016

PLoS Computational Biology Cover Photo, July 2016

Memberships in Professional Societies

Tau Beta Pi

American Institute of Chemical Engineers

Biomedical Engineering Society

Biophysical Society

American Physician Scientists Association

C. Contributions to Science

Throughout my undergraduate and graduate school career, I have conducted mesenchymal stem cell-based heart therapy research using cardiac tissue engineering and systems biology approaches. These experiences have provided the framework to ensure success in my training from this fellowship. A URL to a full list of my published work as found in SciENcv is provided below:

<https://www.ncbi.nlm.nih.gov/myncbi/joshua.mayourian.1/cv/105829/>

I. Undergraduate Research:

Clinical trials have shown encouraging but transient benefits of mesenchymal stem cell therapy on cardiac contractility, underscoring a need to better understand and exploit the underlying mechanisms of mesenchymal stem cell-cardiomyocyte interactions, which predominantly include heterocellular coupling and paracrine signaling. Our bioreactor at the time was unable to isolate paracrine signaling mechanisms from heterocellular coupling, stressing the need for a novel bioreactor. Using my chemical engineering background, I designed an increased throughput multi-tissue bioreactor system for engineered cardiac tissues that could isolate paracrine signaling effects. Isolating the paracrine signaling mechanism enabled Dr. Timothy Cashman and me to identify up-regulated secreted mesenchymal stem cell factors, providing insight into the role of paracrine signaling for potential alternative cardiac therapies. Using my computational background, I also mathematically modeled the paracrine signaling between mesenchymal stem cells and cardiomyocytes to further characterize key biophysical parameters impacting the mesenchymal stem cell-cardiomyocyte secretome.

Abstracts:

Mayourian J, Cashman TJ, Costa KD. Investigating Paracrine Signaling of Mesenchymal Stem Cells for Enhancement of Cardiomyocyte Function. 2012. Biomedical Engineering Society Annual Meeting, Atlanta, Georgia, October 2012. (Abstract for oral presentation)

Cashman TJ, **Mayourian J**, Costa KD. Secretion of Angiogenic and Anti-Apoptotic Factors Accompanies Mesenchymal Stem Cell-Mediated Enhancement of Contractile Function in Engineered Cardiac Tissues. *Circulation Research*, 113(4):A130, August 2013 (Abstract for poster presentation at AHA BCVS meeting).

I. Graduate Research:

Demonstrated benefits of human mesenchymal stem cells (hMSCs) include reduced fibrosis and enhanced contractile function, with predominant mechanisms thought to involve paracrine signaling (PS) and heterocellular coupling (HC) between hMSCs and host myocardium. In our first study, we developed a model of hMSC-cardiomyocyte HC. In the next study, we utilized mathematical modeling and three-dimensional human engineered cardiac tissues (hECTs) to test the hypothesis that hMSC-mediated PS enhances cardiac contractility and minimizes arrhythmogenicity, counterbalancing the unfavorable effects of direct HC. By incorporating such HC and PS effects into an established excitation-contraction model, simulations of hMSC PS-only and combined HC+PS effects on human cardiomyocytes nearly replicated measurements of hECT contractile function under matched experimental treatments. Counteracting PS and HC effects of hMSCs were also revealed in a vulnerable window (VW) analysis of tissue-level arrhythmogenicity in simulated cardiac tissue with moderate and high diffuse fibrosis; hMSC HC+PS conditions had variable effects on VW dependent on the percent of hMSCs delivered, while PS-only conditions consistently decreased the VW, thus minimizing arrhythmogenicity. Together, these findings support our hypothesis, and motivate identifying key hMSC paracrine signaling factors as an alternative cardiac therapy. Recently, we have identified exosomes as the key paracrine signaling factors of hMSC mediated enhancement of hECT contractility.

Publications:

Mayourian J, Savizky RM, Sobie EA, Costa KD (2016) Modeling Electrophysiological Coupling and Fusion between Human Mesenchymal Stem Cells and Cardiomyocytes. *PLoS Comput Biol* 12(7): e1005014. doi:10.1371/journal.pcbi.1005014

Mayourian J, Cashman TJ, Johnson BV, Sachs D, Kaji D, Hare J, Hajjar R, Sobie EA, Costa KD. Human Mesenchymal Stem Cell Paracrine Signaling Counteracts Heterocellular Coupling Effects on Contractility and Arrhythmogenicity. (*submitted*).

Abstracts:

Mayourian J, Savizky RM, Sobie EA, and Costa KD. Modeling Electrophysiological Interactions Between Mesenchymal Stem Cells and Cardiomyocytes for Improved Cell Delivery Cardiotherapeutics. Abstract for Poster Presentation. Biophysical Society Annual Meeting, Los Angeles, CA, February 2016.

Mayourian J, Cashman TJ, Johnson BV, Sachs D, Kaji D, Sobie EA, Costa KD. Human Mesenchymal Stem Cell Paracrine Signaling Counteracts Heterocellular Coupling Effects on Contractility and Arrhythmogenicity. Abstract for Podium Presentation. Biophysical Society Annual Meeting, New Orleans, LA, February 2017.

D. Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
	COOPER UNION (Undergraduate)			COOPER UNION (Undergraduate)	
2010	Computer Programming		2010	Literary Forms and Expressions	
2010	Calculus I		2010	Freshmen Engineering Seminar	
2010	Introduction to Linear Algebra		2010	Engineering Design	
2010	General Chemistry		2011	Texts & Contexts	
2011	Physics I: Mechanics		2011	Engineering Economy	
2011	Calculus II		2011	Making of Modern Society	
2011	Physical Principles of Chemistry		2011	Sophomore Engineering Seminar	
2011	General Chemistry Lab		2012	Microeconomics	
2011	Electromagnetic Phenomena		2013	New Media	
2011	Ordinary and Partial Differential Equations		2013	Process Evaluation & Design I	
2011	Probability and Statistics		2014	The Modern Context	
2011	Material and Energy Balances		2014	Process Evaluation & Design II	
2011	Organic Chemistry I				
2011	Biological Systems				
2012	Optics and Modern Physics				
2012	Complex Variables				
2012	Vector Calculus				
2012	Thermodynamics				
2012	Research Problem I				
2012	Organic Chemistry Lab				
2012	Organic Chemistry II				
2012	Introduction to Neural Biophysics I				
2012	Introduction to Physics Lab				
2012	Fluid Mechanics				
2012	Advanced Thermodynamics				
2012	Research Problem II				
2012	Advanced Organic Chemistry				
2012	Physical Chemistry I				
2012	Instrumental Analysis Lab				
2013	Process Simulation				
2013	Heat and Mass Transfer				
2013	Chemical Reaction Engineering				
2013	Physical Chemistry II				
2013	Introduction to Neural Biophysics II				
2013	Relativity and Electrodynamics				
				ICAHN SCHOOL OF MEDICINE/GRADUATE SCHOOL OF BIOLOGICAL SCIENCES	
			2014	Introduction to Journal Club I	
			2014	Art and Science of Medicine I	
			2014	Medical Scientist Research Seminar	
			2015	Art and Science of Medicine II	
			2015	Medical Scientist Research Seminar	
			2015	Responsible Conduct of Research	
			2015	Introduction to Journal Club II	
			2015	Biophysics and Systems Pharmacology Journal Club	
			2015	Makers Studio I	
			2015	Design, Technology, and Entrepreneurship Directed Reading	
			2016	Makers Studio II	
			2016	Medical Scientist Grand Rounds	
			2016	Biophysics and Systems Pharmacology Journal Club	
			2016	Communication for the Biomedical Entrepreneur	
			2016	Pharmacological Sciences Seminar	

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2013	Basic Principles of Electrical Engineering				
2013	Chemical Engineering Lab I				
2013	Chemical Process Dynamics				
2013	Separation Process Principles				
2014	Materials Science				
2014	Chemical Engineering Lab II				
	COOPER UNION (Graduate)				
2014	Thesis				
2014	Advanced Heat and Mass Transfer				
2014	Thermodynamics of Special Systems				
2013	Advanced Chemical Reaction Engineering				
2013	Convex Optimization Techniques				
2013	Biochemistry				
2012	Bioelectricity				
2011	Bioengineering Applications to Sports Medicine				
	ICAHN SCHOOL OF MEDICINE/GRADUATE SCHOOL OF BIOLOGICAL SCIENCES				
2014	Problem Solving in Biomedical Sciences				
2014	Structures (Anatomy, Histology, and Embryology)				
2014	Molecules, Cells, and Genetics				
2014	Biomedical Sciences I				
2015	Pathology				
2015	Immunology				
2015	Microbiology				
2015	Biomedical Sciences II				
2015	Introduction to Advanced Biostatistics				
2016	Molecular to Systems Pharmacology				
2016	Quantitative Graduate Physiology				
2016	Systems Biology: Biomedical Modeling				
2016	Fundamentals of Nanomedicine				

Undergraduate Cumulative GPA: [REDACTED]; Graduate (Cooper Union) Cumulative GPA: [REDACTED]; Graduate (Icahn School of Medicine at Mount Sinai) GPA: [REDACTED]

All Cooper Union and the Icahn School of Medicine at Mount Sinai courses are graded on a 4.0/4.0 GPA scale. Cooper Union science/engineering courses are graded A, B, C, D, and F. Cooper Union humanities courses are graded A, A-, B+, B, B-, C+, C, C-, D+, D, D-, F. Engineering seminars are graded P (pass) or F (fail).

Icahn School of Medicine at Mount Sinai courses are graded P or F. Passing is a grade of 70 or greater. Certain graduate school courses at the Icahn School of Medicine at Mount Sinai are graded A, B, C, D, and F.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Costa, Kevin David

eRA COMMONS USER NAME (credential, e.g., agency login): KCOSTA

POSITION TITLE: Associate Professor of Medicine (Cardiology)

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	Completion Date MM/YYYY	FIELD OF STUDY
Boston University, Boston MA	B.S.	05/88	Biomedical Engineering
Boston University, Boston MA	M.S.	09/90	Biomedical Engineering
University of California San Diego, La Jolla, CA	Ph.D.	03/96	Bioengineering
The Johns Hopkins University, Baltimore, MD	Postdoctoral	08/97	Atomic Force Microscopy
Washington University, St. Louis, MO	Postdoctoral	08/99	AFM & Cell Mechanics

A. Personal Statement

Dr. Costa will serve as the primary sponsor for this F30 predoctoral MD/PhD training grant. Dr. Costa is a Biomedical Engineer with an established independent research program studying multi-scale cardiovascular mechanobiology from sub-cellular to organ levels, using state-of-the-art experimental and computational techniques including tissue engineering, atomic force microscopy (AFM), elastography imaging, and finite element modeling. As Director of the Cardiovascular Cell and Tissue Engineering program in the Cardiovascular Research Center (CVRC) at the Icahn School of Medicine at Mount Sinai, Dr. Costa is accelerating translational aspects of this research, particularly in the area of high-fidelity screening of gene-, molecular- and cell-based therapies for cardiovascular disease using cells and engineered tissues derived from human pluripotent stem cell sources. Also, as Director of the NIH-funded AFM/confocal Core Facility at Mount Sinai, Dr. Costa is actively fostering collaborative research projects focused on applying atomic force microscopy to new problems in biomedical research. By leading these two research areas at Mount Sinai (cardiac tissue engineering and AFM), Dr. Costa is uniquely positioned to foster interaction with investigators from a variety of departments and fields of expertise, thus providing a rich multi-disciplinary training environment for the Applicant. Dr. Costa has a track record of publications and funding for cardiac tissue engineering and biomechanics research, including a pending NIH R01 (12 percentile) related to the proposed studies. The current F30 proposal reflects his ongoing research interests, continuing existing productive collaborations with Dr. Hajjar, Director of the CVRC at Mount Sinai, and building new relationships with Dr. Sahoo's Laboratory in the CVRC, and Dr. Rice's Computational Biology group at IBM Watson Research, in order to explore human engineered cardiac tissues as a unique screening tool for studying cardiac cell therapies, and elucidating the role of exosomes and their cargo in mesenchymal stem cell-mediated enhancement of cardiac contractile function. Based on his experience and expertise, Dr. Costa is well qualified to serve as the primary mentor on this proposal.

Dr. Costa also has a demonstrated commitment to student mentoring, education, and outreach. In addition to the successful pre-doctoral and postdoctoral students who have graduated from the Costa laboratory, there is currently 1 postdoc, 2 doctoral students, 1 Master's student and 1 post-baccalaureate student in the lab. He has served on 35 domestic and international doctoral thesis committees, and mentored 19 Master's, 30 undergraduate, 5 medical school, and 5 high school student researchers. Dr. Costa is actively involved in mentoring and developing innovative education opportunities for graduate students at Mount Sinai, including a graduate course in Fundamentals of Nanomedicine, and a curriculum in Design, Technology, and Entrepreneurship. He also mentors undergraduate student design teams, and co-directs a summer high school outreach course on Bio-nanotechnology, efforts which focus on providing experiences and opportunities for students from diverse ethnic and socioeconomic backgrounds who are under-represented in science, engineering, and medical professions. These activities provide Dr. Costa with a strong foundation on which to serve as a primary sponsor and mentor for the Applicant, and also will permit the Applicant to gain experience assisting with the teaching and mentoring of less experienced students.

B. Positions and Honors

Positions and Employment

1999-2004	<i>Assistant Professor</i> , Dept. of Biomedical Engineering, Columbia University, New York, NY
2004-2008	<i>Associate Professor</i> , Dept. of Biomedical Engineering, Columbia University, New York, NY
2009-	<i>Associate Professor of Medicine (Cardiology)</i> , Icahn School of Medicine at Mount Sinai, NY, NY
2009-	<i>Director</i> , Cardiovascular Cell and Tissue Engineering Laboratory, ISMMS, New York, NY
2011-	<i>Director</i> , AFM/Confocal Microscopy Departmental Core Facility, ISMMS, New York, NY
2013-2016	<i>co-Director</i> , Graduate Program in Design, Technology and Entrepreneurship, ISMMS, NY, NY
2013-	<i>Scientific co-founder</i> , NovoHeart, Ltd., Hong Kong
2014-2016	<i>Director</i> , Mount Sinai Rapid Prototyping Center, ISMMS, NY, NY

Other Experience and Professional Memberships

1998-	<i>Member</i> , Biomedical Engineering Society
1999-	<i>Member</i> , Biophysical Society
2000-	<i>Member</i> , American Society of Mechanical Engineers
2005-	<i>Professional Member</i> , American Heart Association
2008-	<i>Member</i> , American Physiological Society
2011	<i>ad hoc reviewer</i> , NIH Modeling and Analysis of Biological Systems (MABS) Study Section
2012	<i>ad hoc reviewer</i> , NIH Myocardial Infarction and Metabolism (MIM) Study Section
2014	<i>member</i> , AHA Collaborative Science Award Review Committee
2016	<i>ad hoc reviewer</i> , NIH BTSS and SAT Member Conflict Review Meeting
2016-	<i>ad hoc reviewer</i> , NIH Cardiovascular SBIR/STTR Study Section

Honors

1984-1988	National Society of Professional Engineers Merit Scholarship
1988-	Tau Beta Pi National Engineering Honors Society
1998-1999	NIH Individual National Research Service Award
2000	Faculty Appreciation Certificate, Columbia University
2002, 2006	<i>Invited Delegate</i> , National Academy of Engineering (NAE) Frontiers of Engineering Symposium
2003-2008	National Science Foundation Faculty Early Career Development (CAREER) Award
2004-2007	<i>Editorial Board Member</i> , Biophysical Journal
2007-2014	<i>Associate Editor</i> , Molecular and Cellular Bioengineering
2010-2011	<i>Chair</i> , American Heart Association, Bioengineering Basic Science study section
2010-	<i>co-Chair</i> , ASME Inter-Sector Committee on Federal Research and Development NIH Task Force
2011-	<i>Associate Editor</i> , Journal of Biomechanical Engineering
2013, 2014	<i>One Month Visiting Professorship</i> , Université Pierre et Marie Curie, Paris, France
2016-	<i>Chair</i> , ASME Government Relations Bioengineering Public Policy Task Force

C. Contributions to Science

1. My early research interests were in the field of **cardiac computational modeling**, where I developed and validated state-of-the-art finite element modeling techniques for highly efficient computational analysis of ventricular mechanical function by combining hybrid polynomial interpolation functions with governing equations derived in generalized curvilinear coordinates and solved using prolate spheroidal coordinates that naturally represent the thick-walled ellipsoidal geometry of the LV. The resulting 2-part paper is highly cited in the field of computational cardiac biomechanics, and forms the foundation of finite element models still in use today. We subsequently used these models to validate new techniques in 3D ultrasound elastography, and to develop a patented patient-specific 3D endocardial wall motion analysis technique, with more recent extension to modeling cardiac electrophysiology during mesenchymal stem cell therapy. Related computational modeling concepts were recently published in *Science Signaling* as a teaching resource to broaden their impact for a more generalized audience.
 - a. **Costa KD**, Hunter PJ, Wayne JS, Waldman LK, Guccione JM, McCulloch AD. A three-dimensional finite element method for large elastic deformations of ventricular myocardium--II: prolate spheroidal coordinates. *J Biomech Eng*, 118:464-472, 1996.

- b. Holmes JW, **Costa KD**, Herz S, Ingrassia C, *Methods for Providing Diagnostic Information using Endocardial Surface Data for a Patient's Heart*. Application # 11/801,457 Patent Number: 7,828,735
 - c. **Costa KD**, Kleinstein SH, Hershberg U. Biomedical model fitting and error analysis. *Sci Signal*, Sep 20;4(192):tr9, 2011.
 - d. **Mayourian J**, Savizky RM, Sobie EA, **Costa KD**. Modeling electrophysiological coupling and fusion between human mesenchymal stem cells and cardiomyocytes. *PLoS Comput Biol*. 12(7):e1005014, 2016. (**cover article**)
2. I published a series of papers that established how **3D finite deformations of myocardial laminae** contribute to diastolic, systolic, and residual stress behaviors of ventricular myocardium, challenging the prevailing view of heart muscle as a transversely isotropic tissue. In addition to the large animal experimental data, I also provided the mathematical tools to perform the necessary coordinate transformations and calculate the changes in orientation of muscle fibers and laminar sheets at any point in the cardiac cycle. During this time I published a 3D orthotropic constitutive law describing the mechanical properties of passive myocardium relative to a natural coordinate system defined by the local laminar sheet architecture of the LV wall. In a series of papers by Prof. Peter Hunter's group in New Zealand, this so-called "Costa Law" was rigorously compared to alternative models and determined to be "...the most suitable law to capture the mechanical response of myocardium..." (Schmid et al., 2006, PMID: 16995761). Recently, during my visiting professorship in Paris, this work was extended to model the interlaminar mechanics of the atrial wall, where elevated shear stress was unexpectedly found to cause ion channel trafficking and altered action potentials in atrial myocytes that may explain an increased susceptibility to arrhythmogenesis during hypertrophic remodeling, as published in *PNAS*.
- a. **Costa KD**, Takayama Y, McCulloch AD, Covell JW. Laminar fiber architecture and three-dimensional systolic mechanics in canine ventricular myocardium. *Am J Physiol*, 276:H595-H607, 1999.
 - b. **Costa KD**, Holmes JW, McCulloch AD. Modeling cardiac mechanical properties in three dimensions. *Phil Trans Royal Soc Lond A*, 359:1233-1250, 2001.
 - c. Takayama Y, **Costa KD**, Covell JW. Contribution of the laminar fiber architecture to load dependent changes in the mechanics of the ventricular myocardium. *Am J Physiol Heart Circ Physiol*, 282:H1510-H1520, 2002.
 - d. Boycott HE, Barbier CS, Eichel CA, **Costa KD**, Martins RP, Louault F, Dilanian G, Coulombe A, Hatem SN, Balse E. Shear stress triggers insertion of voltage-gated potassium channels from intracellular compartments in atrial myocytes. *Proc Natl Acad Sci USA*. 110(41):E3955-64, 2013.
3. Pursuing a growing interest in understanding **micro-scale mechanical properties of cardiovascular cells and tissues**, I became an early adopter of the atomic force microscope (AFM) as a tool for imaging and nano-indentation of soft biological samples. I published the first finite element model of AFM indentation, and developed the pointwise modulus technique for detecting depth-dependent variations in elastic properties due to non-linear and heterogeneous properties commonly observed in biological samples but neglected in the standard AFM indentation analysis methods. My lab coined the term "AFM elastography" to describe the method of mapping elastic properties from a high-density array of indentations. While others have pushed the limits of increasing resolution with AFM, my lab has focused on expanding the use of AFM into the meso-scale regime of intact tissue, involving innovations in hardware and software, that has become a critical tool in the rapidly expanding field of mechanobiology. We recently used these techniques to probe the intrinsic micro-mechanical properties of tissues isolated from a transgenic mouse model of Marfan Syndrome, providing new evidence of altered myocardial tissue mechanics that support abnormal extracellular matrix-mediated mechanosignaling in dilated cardiomyopathy, as published in *JCI*, and also demonstrating that prophylactic therapy with Losartan can attenuate Marfans-associated mechanical degradation of aorta and lung tissue.
- a. Azeloglu EU, **Costa KD**. Crossbridge cycling gives rise to spatiotemporal heterogeneity of dynamic subcellular mechanics in cardiac myocytes probed with atomic force microscopy. *AJP Heart Circ Physiol*, 298:H853-860, 2010.
 - b. Azeloglu EU, **Costa KD**. Atomic force microscopy in mechanobiology: measuring microelastic heterogeneity of living cells. *Methods Mol Biol*, 736:303-329, 2011.

- c. Cook JR, Carta L, Bénard L, Chemaly ER, Chiu E, Rao SK, Hampton TG, Yurchenco P; GenTAC Registry Consortium, **Costa KD**, Hajjar RJ, Ramirez F. Abnormal muscle mechanosignaling triggers cardiomyopathy in mice with Marfan syndrome. *J Clin Invest.* 124(3):1329-39, 2014.
- d. Lee J-J, Galatioto J, Rao S, Ramirez F, **Costa KD**. Losartan attenuates degradation of aorta and lung tissue micromechanics in a mouse model of Marfan syndrome. *Ann Biomed Eng*, 44:2994-3006, 2016.
4. My original interest in **cardiac tissue engineering** was to develop a novel *in vitro* model system that could help bridge my micro- and macro-scale mechanics studies, being more physiologic than cells on a Petri dish, but less biologically complex than a natural heart. We published several studies examining the role of boundary conditions on guiding the anisotropy and remodeling of engineered tissues, and developed a variety of constructs including thin sheets of tissue as a cardiac patch, elongated trabecula-like structures for muscle function testing, and higher-order organoid chambers that pump fluid, generate positive stroke work, and allow pressure-volume measurements familiar to cardiologists. Since moving my lab to Mount Sinai in 2009, we have focused efforts on cardiac tissue engineering for modeling genetic and acquired cardiac diseases, and for therapeutic screening applications including drug screening as well as for testing gene- and cell-based therapies and novel nanotherapeutics for cardiac repair. We demonstrated the capability for adult mesenchymal stem cells to significantly enhance the contractile function of ECTs created from neonatal rat cardiocytes, similar to published *in vivo* studies. However, to increase physiologic significance of our findings, the tissue engineering effort in my lab has switched entirely to human pluripotent stem cell derived cardiomyocytes. In one of the first studies to demonstrate human engineered cardiac tissues (hECTs), we measured contractile properties and responses to interventions and systematically compared these with muscle function of natural human myocardium (infant, newborn, adult healthy, and adult failing) to identify similarities and differences and begin to evaluate predictive capabilities of hECTs. The resulting publication was the cover story in a recent issue of *FASEB Journal*, and was featured in a Cutting Edge Medical Technology series on CBSNews.com (www.cbsnews.com/news/scientists-create-beating-heart-tissue-in-a-dish/). My lab has also focused on establishing iPSC-derived hECTs for familial cardiomyopathies, and developing bioreactor systems for non-invasively stimulating and evaluating contractile function during tissue culture, with two patents pending.
- a. Turnbull IC*, Karakikes I*, Serrao GW, Backeris P, Lee J-J, Xie C, Senyei G, Gordon RE, Li RA, Akar FG, Hajjar RJ, Hulot J-S, **Costa KD**. Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium. *FASEB J.* 28(2):644-54, 2014. (**cover article**)
- b. Cashman TJ, Josowitz R, Johnson BV, Gelb BD, **Costa KD**. Human engineered cardiac tissues created using induced pluripotent stem cells reveal functional characteristics of BRAF-mediated hypertrophic cardiomyopathy. *PLoS One.* Jan 19;11(1):e0146697, 2016. (**Editor's Pick: Stem Cell Research**)
- c. Cashman JT, Josowitz R, Gelb BD, Li RA, **Dubois NC**, **Costa KD**. Construction of defined human engineered cardiac tissues to study mechanisms of cardiac cell therapy. *J Vis Exp.* (109), e53447, doi:10.3791/53447, 2016.
- d. Stillitano F*, Turnbull IC*, Karakikes I, Nonnenmacher M, Backeris P, Hulot J-S, Kranias EG, Hajjar RJ, **Costa KD**. Genomic correction of familial cardiomyopathy in human engineered cardiac tissues. *Eur Heart J.* (**in press**).

Complete List of Published Work in My Bibliography (total of 71 publications):

www.ncbi.nlm.nih.gov/sites/myncbi/kevin.costa.1/bibliography/45727371/public/?sort=date&direction=ascending

D. Research Support

Active Research Support

NHLBI R01 HL115195	(H Qiu)	03/01/13 - 02/28/18
<i>Intrinsic Stiffness of Aortic Vascular Smooth Muscle Cell in the Development of Hypertension</i>		
Overall goal to determine alterations in aortic stiffness due to novel hypothesis that a key component occurs intrinsic to vascular smooth muscle cells (VSMCs).		
Role: co-PI of sub-contract		
NHLBI R01 HL113499 (B Gelb and I Lemischka) 07/01/13 - 06/30/17		
<i>Human Induced Pluripotent Cell Models of Pediatric Cardiac Disorders</i>		

Overall goal to use human iPSC lines from infants and children with cardiac muscle abnormalities due to inherited disorders of the RAS/MAPK signaling pathway to elaborate the pathogenesis of pediatric heart disorders and inform development of novel therapeutic approaches and clinical care pathways.

Role: co-Investigator

NHLBI R01 HL126173

(F Ramirez)

07/01/15-06/30/20

Characterization of a Novel Pathway of Aortic Aneurysm Formation

Overall goal to advance knowledge of molecular mechanisms driving aortic aneurysms in a transgenic mouse model of the human disease, toward development of more effective, evidence based therapies.

Role: co-Investigator

NIH U54OD020353

(R Cagan)

09/01/15-08/31/20

A New Disease Platform Leveraging Complex Drosophila and Mammalian Models

Project III of this Specialized Center grant, titled “Creating a platform linking Drosophila, mouse, human iPS, and human tumor tissue to generate personalized therapeutics for cancer and RASopathies”, will use a novel approach to discover mutation-specific therapy for RASopathy-associated HCM, currently an untreatable condition, using fly avatar-generated leads to test in mutant human cardiomyocytes.

Role: co-Investigator

Sponsored Research Agreement, NovoHeart Ltd. (Costa)

05/01/16 - 04/31/17

Screening Platforms Based on Human Engineered Cardiac Tissues

Two goals of this sponsored research from NovoHeart, Ltd. are 1) develop and validate human engineered cardiac tissues (hECTs) as next-generation screening tools for drug discovery and toxicity applications, and 2) design and develop custom bioreactors for culturing, stimulating, and testing hECT contractile function.

Role: PI

Recently Completed Research Support

NHLBI F30 HL118923

(TJ Cashman)

09/02/14 - 7/01/16

Human Cardiac Tissue Engineering for Stem Cell-Based Therapeutic Discovery

The aims of this proposal are to investigate the relative contributions of 1) direct cell-cell contact and 2) indirect paracrine signaling effects of therapeutic mesenchymal stem cells (MSCs) to enhance cardiomyocyte function in human engineered cardiac tissues. Dr. Costa served as the sponsor for this F30 training grant.

Role: Sponsor

NYSTEM N13G-171

(N Dubois)

06/01/14 – 05/31/16

Exploring Pathophysiology and Therapy Strategies of Muscular Dystrophy-related Cardiac Dysfunction with Patient-specific Induced Pluripotent Stem Cells

Overall goal to establish a human disease model for Duchenne Muscular Dystrophy (DMD) and characterize the specific cardiac defects in human cells, to understand how the dystrophin gene mutations affect human heart tissue formation and function, and to improve treatment options for DMD patients.

Role: co-Investigator

NHLBI N01 contract HHSN268201000045C (Z Fayad and R Langer)

08/13/10 – 08/12/15

Translational Nanomedical Therapies for Cardiac & Vascular Diseases

The overall goal of this Program of Excellence in Nanotechnology (PEN) contract is the development and imaging-facilitated evaluation of translational nanotechnology-based tools, and the development of associated multidisciplinary trainees, focused on the treatment of heart failure and atherosclerosis. Co-investigator on one project to investigate minimally invasive nanotechnology-based approaches to deliver regeneration factors to the infarcted myocardium, specifically using engineered human cardiac tissues for pre-clinical testing.

Role: co-Investigator

NHLBI R21 HL095980

(KD Costa)

02/05/10-01/31/12

Engineered Cardiac Niche Arrays for Exploring and Optimizing Stem Cell Therapies

The goal of this grant was to develop innovative new tools and approaches combining soft lithography and tissue engineering, with the overall objective of understanding and directing stem cell differentiation for cardiac repair applications.

Role: PI

BIOGRAPHICAL SKETCH

NAME Roger J. Hajjar	POSITION TITLE Professor of Medicine Director, Cardiovascular Research Center		
eRA COMMONS USER NAME rjhajjar			
EDUCATION/TRAINING (<i>Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.</i>)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Johns Hopkins University Harvard University	BS MD	1986 1990	Biomedical Engineering Medicine

A. Personal Statement

I am pleased to serve as a co-mentor for this F30 NRSA for Joshua Mayourian. I have had the opportunity to mentor over fifty post-doctoral fellows and graduate students since starting my laboratory at the Cardiovascular Research Center at Massachusetts General Hospital in 1998, with many fellows on NIH training grants. Since assuming my position at Mount Sinai as director of the Cardiovascular Research Center, previously serving as director of the Cardiovascular Fellowship Program, and currently serving as co-PI on a T32 Training Grant, I have focused more specifically on trainees and their mentorship while providing an interactive environment for scientists and physician-scientists pursuing research elucidating molecular mechanisms of cardiovascular diseases. My laboratory has had extensive experience studying cardiac physiology in both in vitro and in vivo models with a long-standing research program targeting signaling pathways in cardiac hypertrophy and heart failure. My group has developed a number of methodologies for cardiac gene transfer and these techniques have been adopted by a large number of investigators in the field. My laboratory has had a specific interest in defining the various genes that are involved in cardiac hypertrophy and in the transition to heart failure. My work over the past fifteen years has been focused on the development of gene-based therapies for heart failure, with special emphasis on the SERCA-PLN system. One of our major efforts over the past decade has been to use AAV vectors to over-express SERCA2a in experimental models that resulted in the CUPID clinical trials. Even though the results of the large international Phase 2b trial assessing AAV1.SERCA2a in patients with heart failure did not show any beneficial effects, there is a strong consensus in the biomedical community that correction of calcium cycling is an extremely promising avenue and other approaches require further investigation. My laboratory has a specific focus on gene therapy in various animal models (both rodents and large/pre-clinical) of cardiovascular diseases, including strategies to harness and amplify endogenous and stem cell based cardiac repair mechanisms. Since I recruited Dr. Costa to the CVRC in 2009, we have developed a productive collaborative relationship, and we will work together to provide the Applicant with the support needed to successfully achieve the aims of his proposal. In addition, I bring the perspective of a physician-scientist who has successfully translated work from the laboratory into new therapies for the clinic, and I hope to provide the Applicant with insight and advice that I wish I had received as I began my career.

B. Positions & Honors**Positions & Employment**

- 1990-93: Intern, & Resident, Medical Services, Massachusetts General Hospital
- 1993-1996: Clinical & Research Fellow in Cardiology, Massachusetts General Hospital
- 1997-2006: Principal Investigator, Cardiovascular Research Center, Massachusetts General Hospital
- 1998-2002: Assistant Professor in Medicine, Harvard Medical School
- 2002-6: Director of the Cardiovascular Laboratory of Integrative Physiology and Imaging, MGH
- 2003-6: Associate Professor of Medicine, Harvard Medical School
- 2007-: Arthur & Janet C. Ross Professor of Medicine, Mount Sinai School of Medicine,
- 2007-: Director of the Cardiovascular Research Center, Mount Sinai School of Medicine, NY.

Professional Memberships

- 2012: Co-Chair AHA BCVS
- 2009-12: Member, NIH CCHF Study Section
- 2012-14: Chair, NIH CCHF Study Section
- 2013-: American Association of Physicians.

2014-: Associate Editor, JACC

Honors

1983-1986: Johns Hopkins University: Dean's list; Premedical Honor Society, Engineering Honor Society Tau Beta Pi, University & Departmental Honors at graduation
1986: Biomedical Engineering Research Award; Johns Hopkins University
1990: Cum Laude at graduation, Harvard Medical School
1993: Calderwood Research Award, Massachusetts General Hospital
1995: Roman DeSanctis Clinical Scholar Award, Massachusetts General Hospital
1996-97: Paul Dudley White Fellowship Award, American Heart Association, Massachusetts Affiliate
1996: Winner, Melvin L. Marcus Young Investigator Award, American Heart Association (1996);
1996-2001: Mentored Clinician Scientist Award, NIH
1997-2001: FIRST AWARD, NIH
1999: First Prize, AstraZeneca Cardiovascular Young Investigators Forum
1999: Doris Duke Clinical Scientist Award
2001: Paul Beeson Scholar Award, American Federation for Aging Research
2002: Henry N. Neufeld Memorial Award
2005: American Society for Clinical Investigation
2007: The Arthur & Janet Ross Chair in Medicine
2011: Johns Hopkins University Distinguished Alumni Award
2012: Dean's Award for Excellence in Translational Science, Mount Sinai School of Medicine
2013: AHA Basic Science Achievement Award
2013: ACC NY Paul Dudley Lecture

C. Contributions to Science

1. My early publications focused on the role of intracellular calcium abnormalities in the pathophysiology of heart failure. Over the years my laboratory has validated the cardiac isoform of the sarcoplasmic reticulum Ca^{2+} ATPase pump (SERCA2a) and its regulators as important determinants of contractile dysfunction in heart failure. We have used gene transfer to increase the expression of SERCA2a and knock down its endogenous regulator phospholamban to demonstrate the importance of SERCA2a/phospholamban complex as a target for the treatment of heart failure.
 - a. Gwathmey, J.K., **Hajjar, R.J.** (1990) Relation between steady-state force and intracellular $[\text{Ca}^{2+}]$ in intact human myocardium. Index of myofibrillar responsiveness to Ca^{2+} . *Circulation*. 82:1266-1278.
 - b. del Monte, F., Harding, S.E., Schmidt, U., Matsui, T., Kang, Z.B., Dec, G.W., Gwathmey, J.K., Rosenzweig, A., **Hajjar, R.J.** (1999) Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* 100:2308-2311.
 - c. Miyamoto, M.I., del Monte, F., Schmidt, U., DiSalvo, T.S., Kang, Z.B., Matsui, T., Guerrero, J.L., Gwathmey, J.K., Rosenzweig, A., **Hajjar R.J.** (2000) Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *PNAS*. 97:793-798.
 - d. del Monte, F., Harding S.E., Dec G.W., Gwathmey J.K., **Hajjar R.J.** (2002) Targeting phospholamban by gene transfer in human heart failure. *Circulation*.105:904-907.
2. My laboratory has focused on developing a number of techniques for gene transfer to alter the expression of specific genes in the myocardium of rodent and pre-clinical cardiovascular models. This has led to the adaptation of these gene transfer techniques in clinical trials. More recently, we have used genome editing in iPS induced cardiomyocytes to correct phospholamban Arginine deletion mutation using TALEN and CRISPR.
 - a. **Hajjar RJ**, Schmidt U, Matsui T, Guerrero JL, Lee KH, Gwathmey JK, Dec GW, Semigran MJ, Rosenzweig A (1998). Modulation of ventricular function through gene transfer in vivo. *PNAS*.95:5251-5256.
 - b. Kawase Y, Ly HQ, Prunier F, Lebeche D, Shi Y, Jin H, Hadri L, Yoneyama R, Hoshino K, Takewa Y, Sakata S, Peluso R, Zsebo K, Gwathmey JK, Tardif JC, Tanguay JF, **Hajjar RJ**.(2008) Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *JACC* 51:1112-1119.
 - c. Hayase M, Del Monte F, Kawase Y, Macneill BD, McGregor J, Yoneyama R, Hoshino K, Tsuji T, De Grand AM, Gwathmey JK, Frangioni JV, **Hajjar RJ**. (2005) Catheter-based antegrade intracoronary

- viral gene delivery with coronary venous blockade. *American Journal of Physiology Heart and Circulatory Physiology.* 288:H2995-3000.
- d. Karakikes I, Stillitano F, Nonnenmacher M, Tzimas C, Sanoudou D, Termglinchan V, Kong CW, Rushing S, Hansen J, Ceholski D, Kolokathis F, Kremastinos D, Katoulis A, Ren L, Cohen N, Gho JM, Tsipras D, Vink A, Wu JC, Asselbergs FW, Li RA, Hulot JS, Kranias EG, **Hajjar RJ.** (2015) Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy. *Nature Communications.* 6:6955.
doi:10.1038/ncomms7955
3. We have developed a number of important models of heart failure in small and large animal models to further our pre-clinical work. These models include models of i) myocardial infarction induced heart failure, ii) mitral regurgitation induced volume overload, iii) aortic banding induced diastolic dysfunction, iv) Pulmonary hypertension model using proximal pulmonary artery coiling and distal embolization or post-capillary pulmonary hypertension. These models have been useful in testing various treatment interventions
- Chen J, Chemaly ER, Liang LF, LaRocca TJ, Yaniz-Galende E, **Hajjar RJ.** (2011) A new model of congestive heart failure in rats. *AJP Heart and Circulatory Physiology* 301:H994-1003
 - Ishikawa K, Aguero J, Tilemann L, Ladage D, Hammoudi N, Kawase Y, Santos-Gallego CG, Fish K, Levine RA, **Hajjar RJ.** (2014) Characterizing preclinical models of ischemic heart failure: differences between LAD and LCx infarctions. *AJP Heart and Circulatory Physiology* 307:H1478-1486.
 - Aguero J, Ishikawa K, Hadri L, Santos-Gallego C, Fish K, Hammoudi N, Chaanine A, Torquato S, Naim C, Ibanez B, Pereda D, Garcia-Alvarez A, Fuster V, Sengupta PP, Leopold JA, **Hajjar RJ.** (2014). Characterization of right ventricular remodeling and failure in a chronic pulmonary hypertension model. *AJP Heart and Circulatory Physiology.* 307:H1204-1215.
 - Ishikawa, K., Fish, K., Aguero, J., Yaniz-Galende, E., Jeong, D., Kho, C., Tilemann, L., Fish, L., Liang, L., Eltoukhy, A.A., Anderson, D.G., Zsebo, K., Costa, K., **Hajjar, R.J.** (2015). Stem cell factor gene transfer improves cardiac function after myocardial infarction in swine. *Circulation Heart Failure* 8, 167-174.
4. We have initiated and successfully completed Phase 1 and Phase 2 First-in-Man clinical trials in gene therapy for patients with severe heart failure. These clinical trials have been the direct consequence of our work validating SERCA2a as a critical target in heart failure.
- Jaski BE, Jessup ML, Mancini DM, Cappola TP, Pauly DF, Greenberg B, Borow K, Dittrich H, Zsebo KM, **Hajjar RJ.** (2009) Calcium Up-Regulation by Percutaneous Administration of Gene Therapy In Cardiac Disease Trial I. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *Journal of Cardiac Failure.* 15:171-181.
 - Jessup M, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, **Hajjar RJ.** (2011) Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease I. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca²⁺-ATPase in patients with advanced heart failure. *Circulation.* 124:304-313.
 - Zsebo K, Yaroshinsky A, Rudy JJ, Wagner K, Greenberg B, Jessup M, **Hajjar RJ.** (2014) Long-term effects of AAV1/SERCA2a gene transfer in patients with severe heart failure: analysis of recurrent cardiovascular events and mortality. *Circulation Research.* 114:101-108.
 - Greenberg, B., Yaroshinsky, A., Zsebo, K.M., Butler, J., Felker, G.M., Voors, A.A., Rudy, J.J., Wagner, K., and **Hajjar, R.J.** (2014). Design of a phase 2b trial of intracoronary administration of AAV1/SERCA2a in patients with advanced heart failure: the CUPID 2 trial (calcium up-regulation by percutaneous administration of gene therapy in cardiac disease phase 2b). *JACC Heart Failure* 2, 84-92
5. More recently, we found that the levels and activity of SERCA2a in cardiomyocytes are modulated in parallel with the levels of a cytoplasmic protein, small ubiquitin-like modifier type 1 (SUMO1). We found that SERCA2a and SUMO1 levels were both reduced in models of heart failure and in failing human myocardium. We also found that SERCA2a is SUMOylated at lysine sites 480 and 585 and that this SUMOylation is responsible for stabilizing SERCA2a and enhancing its activity. Furthermore, we showed

that increasing SUMO1 levels led to restoration of SERCA2a levels, improved hemodynamic performance, and reduced mortality in a murine model of HF. We have undertaken a small molecule screen and identified through sequential screening small molecules that specifically increase the SUMOylation of SERCA2a.

- a. Kho C, Lee A, Jeong D, Oh JG, Chaanine AH, Kizana E, Park WJ, **Hajjar RJ**. (2011) SUMO1-dependent modulation of SERCA2a in heart failure. *Nature*.477:601-605.
- b. Tilemann L, Lee A, Ishikawa K, Aguero J, Rapti K, Santos-Gallego C, Kohlbrenner E, Fish KM, Kho C, **Hajjar RJ**. SUMO-1 gene transfer improves cardiac function in a large-animal model of heart failure. *Science Translational Medicine*. 2013;5:211ra159.
- c. Lee, A., Jeong, D., Mitsuyama, S., Oh, J.G., Liang, L., Ikeda, Y., Sadoshima, J., Hajjar, R.J., and Kho, C. (2014). The role of SUMO-1 in cardiac oxidative stress and hypertrophy. *Antioxidants & Redox Signaling* 21, 1986-2001.
- d. Kho C, Lee A, Jeong D, Oh JG, Gorski PA, Fish K, Sanchez R, DeVita RJ, Christensen G., Dahl R. **Hajjar RJ**. (2015) Small Molecule Activation of SERCA2a SUMOylation for the Treatment of Heart Failure. *Nature Communications* 6:7967 doi:10.1038/ncomms8229

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/roger.hajjar.1/bibliography/41154049/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

P50 HL112324 Hajjar (PI) 04/01/12-03/31/17

TRIP: Targeted Gene Therapy for the Treatment of Heart Failure

The goal of this study it to carry out a Phase1, Open-Labeled, Dose-Escalation Trial of BNP111.CMV.I1c by Intra-Coronary Infusion in patients with heart failure followed by a Phase 2, Randomized, Double-Blinded, Placebo-Controlled Dose Escalation Trial of Intra-Coronary Infusion of BNP111.sc-CMV.I1c in patients with heart failure.

Role: PI

R01 HL119046 Hajjar/Akar (PI) 01/02/14-12/31/17

Targeting Abnormal Calcium Cycling Using Novel Gene Therapy Vectors

The goal of this project it to examine the role of altering calcium cycling proteins by gene transfer in pre-clinical models of heart failure.

Role: PI

R01 HL117505 Hajjar (PI) 07/01/13-06/30/18

SUMO1 and SERCA2a Function

This project will examine the role of SUMO1 in the setting of heart failure.

Role: PI

T32 HL007824 Gelb/Hajjar (Co-PI) 08/01/13-07/31/18

Training Program in Molecular and Cellular Cardiology

This training program seeks to train physician-scientists and postdoctoral fellows in molecular and cellular cardiology.

Role: Co-PI

Fondation Leducq 10/01/13-09/30/18

Cellular and Molecular Targets to Promote Therapeutic Cardiac Regeneration

This project brings together both established and younger scientists who study cardiac stem cell biology, cardiac de-differentiation, cardiac gene transfer and clinical heart disease to jointly address cellular and molecular processes contributing to cardiac repair.

Role: Director, Core Project

R01 HL128072 Hajjar/Mercola (PI) 04/01/15-03/31/20

Role of miR25 in Heart Failure

The goal of this project is to investigate the role of a non-coding microRNA (miR25) in failing hearts.

Role: PI

R01HL129814 Thomas/Hajjar (PI) 07/01/15- 06/30/20

Calcium Pump Activators for Heart Failure Therapy

In this proposal, we will test small molecules generated at the University of Minnesota in single isolated cardiac myocytes and in murine and porcine models of myocardial infarction induced heart failure.

Role: Co-Investigator

1R01HL128099 Levine/Hajjar 12/15/15-11/30/19

Treating Ventricle and Valve: New Synergies for Ischemic LV Remodeling with MR

In this proposal, we will be involved in developing the viral vectors necessary for the completion of the application. Specifically, AAV vectors encoding for CCN5 and SERCA2a will be developed.

R01HL131404 Hajjar/Weber 07/01/16-06/30/21

Anti-AAV Antibodies as an Obstacle to Cardiac AAV Gene Therapy

The goals of this project are 1) to determine the effect of neutralizing antibodies on the treatment of heart failure with AAV1.SERCA2a, 2) to determine the major epitopes of the cardiotropic AAVs, AAV1, AAV6 and AAV6 and to isolate cardiotropic AAVs with increased resistance to pre-existing neutralizing antibodies and 3) to develop plasmapheresis with AAV-columns to deplete neutralizing antibodies from the blood of patients.

R01 HL113497 Hulot (PI) 04/01/12-02/28/17

Role of STIM1 in cardiac hypertrophy and heart failure

This study will evaluate the role of STIM1 in cardiac hypertrophy and heart failure.

Role: Co-Investigator

1R01HL130423-01 Kovacic 01/25/16-12/31/20

Toward Therapeutic Manipulation of Endothelial to Mesenchymal Transition

This proposal seeks to leverage several novel discoveries made by our team to define the core biologic mechanisms of EndMT in atherosclerosis and cardiovascular disease, to understand how we can manipulate EndMT to promote healing after vascular grafting procedures, and ultimately to develop novel therapeutic strategies for cardiovascular disease.

Role: Co-Investigator

HL105826 Sadayappan 04/01/16-03/31/21

Cardiac myosin binding protein-C: Structure and Function

In this proposal, we will work with Dr. Sadayappan and his lab members, Dr. Govindan and Mr. Lin, in assisting with vector design, injection protocol and systematic delivery of AAV9 particles, as well as the validation of the results.

1R01HL133554-01 08/01/2016 – 07/31/2021

Interactions of SERCA2a and BMPRII in Vascular Disease

Our goal is to attenuate pulmonary vascular remodeling by using SERCA2a gene therapy as a therapeutic approach to restore BMPR2 and reverse the pathological changes in PAH.

Completed Research Support

268201000045C Fayad (PI) 09/01/10-08/31/15

Program of Excellence in Nanotechnology (PEN): Translational Nanomedical Therapies for Cardiac and Vascular Diseases

This project will use a nanotechnology-based approach to develop strategies to deliver novel therapeutic agents to atherosclerotic plaques.

Role: Co-Director, Project 1

R01 HL088434 Hajjar (PI) 03/01/10-11/30/13

Gene Therapy with Cardiotropic Vectors for the Treatment of Heart Failure

The goal of this grant was to target protein phosphatase 1 in large animal models of heart failure using novel cardiotropic vectors.

Role: PI

PHS Fellowship Supplemental Form**Introduction**

1. Introduction
(RESUBMISSION) Response to Reviewers Final.pdf

Fellowship Applicant Section

2. Applicant's Background and Goals for Fellowship Training* Applicants Background and Goals for Fellowship Training Final.pdf

Research Training Plan Section

3. Specific Aims* Specific Aims Final.pdf
 4. Research Strategy* Research Strategy Final.pdf
 5. Respective Contributions* Respective Contributions Final.pdf
 6. Selection of Sponsor and Institution* Selection of Sponsor and Institution Final.pdf
 7. Progress Report Publication List
(RENEWAL)
 8. Training in the Responsible Conduct of Research* Responsible Conduct of Research Final.pdf

Sponsor(s), Collaborator(s) and Consultant(s) Section

9. Sponsor and Co-Sponsor Statements Sponsor Information Final.pdf
 10. Letters of Support from Collaborators, Contributors and Consultants Letters of Support Final.pdf

Institutional Environment and Commitment to Training Section

11. Description of Institutional Environment and Commitment to Training INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING Final.pdf

Other Research Training Plan Section**Human Subjects**

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? Yes No

12. Human Subjects Involvement Indefinite?
 13. Clinical Trial?
 14. Agency-Defined Phase III Clinical Trial?
 15. Protection of Human Subjects
 16. Data Safety Monitoring Plan
 17. Inclusion of Women and Minorities
 18. Inclusion of Children

Vertebrate Animals

The following item is taken from the Research & Related Other Project Information form and repeated here for your reference. Any change to this item must be made on the Research & Related Other Project Information form.

Are Vertebrate Animals Used? Yes No

19. Vertebrate Animals Use Indefinite?

PHS Fellowship Supplemental Form

20. Are vertebrate animals euthanized?

If "Yes" to euthanasia

Is method consistent with American Veterinary
Medical Association (AVMA) guidelines?

If "No" to AVMA guidelines, describe method and
provide scientific justification

21. Vertebrate Animals

Other Research Training Plan Information

22. Select Agent Research

23. Resource Sharing Plan

Resource Sharing Plan Final.pdf

24. Authentication of Key Biological and/or Chemical
Resources

PHS Fellowship Supplemental Form**Additional Information Section****25. Human Embryonic Stem Cells**

Does the proposed project involve human embryonic stem cells?* Yes No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s), using the registry information provided within the agency instructions. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

0061			

26. Alternate Phone Number:

27. Degree Sought During Proposed Award:

Degree: If "other", please indicate degree type: Expected Completion Date (month/year):
OTH: Other MD/PhD 07/2022

28. Field of Training for Current Proposal*: 306 Bioengineering & Biomedical Engineering

29. Current Or Prior Kirschstein-NRSA Support?* Yes No

If yes, please identify current and prior Kirschstein-NRSA support below:

Level*	Type*	Start Date (if known)	End Date (if known)	Grant Number (if known)
Predoctoral	Institutional	07/01/2014	06/30/2015	5T32GM007280
Predoctoral	Individual	07/01/2016	06/30/2017	T32GM062754
.....

30. Applications for Concurrent Support?* Yes No

If yes, please describe in an attached file:

31. Citizenship*

U.S. Citizen U.S. Citizen or Non-Citizen National? Yes No

Non-U.S. Citizen With a Permanent U.S. Resident Visa
 With a Temporary U.S. Visa

If you are a non-U.S. citizen with a temporary visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, please also check here.

Name of Former Institution:*

32. Change of Sponsoring Institution

PHS Fellowship Supplemental Form**Budget Section****All Fellowship Applicants:**

1. Tuition and Fees*:

None Requested Funds Requested

Year 1	\$18,700.00
Year 2	\$18,700.00
Year 3	\$55,947.00
Year 4	\$58,117.00
Year 5	\$0.00
Year 6 (when applicable)	\$0.00
Total Funds Requested:	\$151,464.00

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary:	Amount	Academic Period	Number of Months		
3. Stipends/Salary During First Year of Proposed Fellowship:					
a. Federal Stipend Requested:	Amount	Number of Months			
b. Supplementation from other sources:	Amount	Number of Months			
Type (sabbatical leave, salary, etc.)					
Source					

Appendix

Introduction to the Resubmission Application:

I thank the reviewers for their insightful and helpful critique of the initial F30 application, especially the suggestion to perform more hypothesis-driven research. Over the past months, we have generated exciting preliminary data that motivates our hypothesis that exosomal miRNA-21 is essential for human mesenchymal stem cell (hMSC)-paracrine mediated enhancement of cardiac contractility. Although phenotypic immaturity of human embryonic stem cell derived-cardiomyocytes remains a challenge in the field, our lab has continued to make progress toward improved maturation and disease-specific modeling. Recently, our lab has published two studies where human induced pluripotent stem cell-derived human engineered cardiac tissues (hECTs) were able to model key functional characteristics of familial hypertrophic and dilated cardiomyopathies. We have revised our rationale and disease models accordingly to address this timely research topic; to this end, the hECT in vitro model is more suitable for studying therapies for non-ischemic dilated cardiomyopathy (NIDCM), a focus of our resubmission. We hope you will find this revised application to be responsive to the original reviews. We feel it was considerably improved as a result of the process. Due to the extensive nature of the revisions, specific changes are not highlighted. Responses to individual comments are provided below:

Critique 1:

- As a source of independent funding, my Sponsor, Dr. Costa, received a percentile score of 12% as PI of an NHLBI R01 directly related to this F30 topic, with funding expected to begin in April 2017.
- Based on new preliminary data, we are able to form a hypothesis that exosomal miRNA-21 is essential for hMSC-paracrine mediated enhancement of cardiac contractility. This addresses the “absence of hypothesis testing” in our previous application. Our preliminary data shows that: 1) exosomes are necessary and sufficient for hMSC-paracrine mediated enhancement of hECT developed force; 2) hMSC exosomal factors enhance hECT sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), L-type calcium channel (LTCC), and anti-apoptotic gene expression; and 3) miRNA-21 is a lead factor of exosomal cargo responsible for such effects based on qPCR and our partial least squares regression mathematical model.
- While the “established consensus in the field” is that “cell engraftment benefits for heart function are derived by paracrine factors”, to our knowledge, no study has identified the key inotropic factors responsible. Our focus on key hMSC inotropic factors, such as exosomal miRNA-21, provides an innovative and alternative therapeutic approach for hMSC-based NIDCM therapies.
- Regarding limitations based on the “dubious correspondence of hECT to altered function of host heart in response to engrafted tissue” and “modeling ventricular function with a spontaneously beating preparation”, we suggest that hECTs offer a system that is more physiologic than cells on a Petri dish, but less complex than a natural heart. Of course, the hECT model does have limitations, but also has important species-specific advantages. Also, the use of our recently established iPSC-derived hECT model with R14-del phospholamban mutation is well suited to the new focus on treatment of NIDCM.
- We refocus our efforts from “unclear” computational approaches to methodical hypothesis-driven research to identify the role of miRNA-21 in hMSC exosome-mediated enhancement of hECT function.
- Dr. Hajjar has provided significant mentoring and support of the applicant during the past year, including weekly interactions at the department-wide journal club, essential feedback for this F30 application, co-authorship on the applicant’s manuscript under submission to *Circulation Research*, and departmental funds to help support the applicant’s research.

Critique 2:

- Phenotypic immaturity of stem cell-derived cardiomyocytes continues to present challenges in the field, which we acknowledge and attempt to address as stated above.
- Sub-physiologic contractile forces may present a major roadblock for hECT applications aimed at surgical repair of damaged heart muscle, but for in vitro screening applications as proposed herein, we would argue that the force amplitude is less critical than the qualitative characteristics of the hECT function, which we now show to mimic several key features of familial NIDCM vs. healthy controls.

Critique 3:

- The concern about Sponsor funding has been addressed, as stated in response to Critique 1.
- By generating exciting new preliminary data, we are able to form a specific hypothesis that exosomal miRNA-21 is essential for hMSC-paracrine mediated enhancement of cardiac contractility. This addresses the “lack of clear hypotheses” noted in our previous application, as described in the response to Critique 1.
- Our Resource Sharing Plan has been edited to appropriately address “the issue of sharing of computational models and other knowledge gained during the study.”

DOCTORAL DISSERTATION AND RESEARCH EXPERIENCE

Citations for all referred papers and abstracts can be found in the Fellowship Application Biographical Sketch.

Ruben M. Savizky, PhD, Cooper Union, New York, NY

2012

Synthesizing Substituted Guanidines for HIV Therapeutics

As a sophomore at Cooper Union, I conducted organic chemistry research with Professor Ruben Savizky as my mentor. Prior to winter break, I overheard a conversation between Master's and undergraduate students discussing the difficulty of synthesizing substituted guanidines. My investigative nature led me to their mentor, Professor Savizky. As I sat in his office as he elucidated the background of the work: having received his PhD in chemistry, his dissertation entailed a biophysical approach to understand RNA structure. Using his engineering background, he has transformed his thesis work into translational research for inhibiting HIV-1 transcription. Immediately, I became intrigued with the possibility of direct effects on HIV-1 patients. The Tat-TAR interaction is involved in transcriptional initiation and elongation of HIV-1. Therefore, molecules that inhibit the Tat-TAR interaction are of special interest to hinder transcription of HIV-1. The guanidinium functional group has the electric properties to possibly inhibit Tat-TAR interaction due to the highly negative charge. This research seeks to discover the conditions that would optimize percent yield of substituted guanidines. I succeeded in synthesizing substituted guanidines by using microwave irradiation. The technique I utilized was used by the Master's student, which led to his thesis. To calculate the percent yield of these substituted guanidines, we analyzed the output NMR spectra. Future work included testing the ability of substituted guanidines to inhibit Tat-TAR interaction, with hopes for clinical application.

Kevin D. Costa, PhD, Icahn School of Medicine at Mount Sinai, New York, NY

2012

The Role of Paracrine Signaling in Mesenchymal Stem Cell-Mediated Enhancement of Cardiac Function

Mentored by Dr. Kevin Costa and then-graduate student Dr. Timothy Cashman, I investigated mechanisms whereby mesenchymal stem cells (MSCs) improve cardiomyocyte function. The specific mechanism under investigation was paracrine signaling, or indirect cell-to-cell signaling through secreted factors. In the course of this study, Dr. Costa and I recognized the need for a more efficient bioreactor to construct engineered cardiac tissues. Taking the whole lab's objectives into consideration, I designed a low cost, increased efficiency bioreactor prototype useful for multiple lab members; in fact, I currently use the final version of this bioreactor system in my thesis work to construct engineered cardiac tissue from human cardiomyocytes. Using ELISA, I also helped determine the concentration of soluble factors (specifically Vascular Endothelial Growth Factor) in the media of co-cultured MSCs and cardiomyocytes. My yearning to predict an outcome using math and physics skills led me to construct a model of what we should expect, requiring me to independently acquire graduate level knowledge about biotransport phenomena. Applying transport theory, I constructed a model of the concentration of the VEGF released into the media and consumed over time, correlating this with measurements of the contractile force of the engineered cardiac tissue during electrical pacing. I presented this work at the Biomedical Engineering Society Annual Meeting in 2012 as a podium presentation. This also led to an abstract co-authorship in Circulation Research.

Ruben M. Savizky, PhD and Kevin D. Costa, PhD

2013-2016

Master's Thesis: Modeling Electrophysiological Interactions Between Mesenchymal Stem Cells and Cardiomyocytes for Improved Cell Delivery Cardiotherapeutics

I took the lead role on mathematically modeling the electrophysiological consequences of direct MSC-cardiomyocyte coupling, which ultimately became my Master's thesis work. Human mesenchymal stem cell (hMSC) delivery has demonstrated promise in preclinical and clinical trials for myocardial infarction therapy; however, broad acceptance is hindered by limited understanding of hMSC-human cardiomyocyte (hCM) interactions. To better understand the electrophysiological consequences of direct heterocellular connections between hMSCs and hCMs, three original mathematical models were developed, representing an experimentally verified triad of hMSC families with distinct functional ion channel currents. Substantial variations in action potential waveform—such as decreased action potential duration (APD) and plateau height—were found when hCMs were coupled to the two hMSC models expressing functional delayed rectifier-like human ether à-go-go K⁺ channel 1 (hEAG1); the effects were exacerbated for fused hMSC-hCM hybrid cells. The third family of hMSCs (Type C), absent of hEAG1 activity, led to smaller single-cell action potential alterations during coupling and fusion, translating to longer tissue-level mean action potential wavelength. In a simulated 2-D monolayer of cardiac tissue, re-entry vulnerability with low hMSC insertion was approximately

eight-fold lower with Type C hMSCs compared to hEAG1-functional hMSCs. Overall, this study provides novel electrophysiological models of hMSCs and predicts possible arrhythmogenic effects of hMSCs when heterocellularly coupled to healthy hCMs. This led to my Master's thesis, and a first author manuscript published in *PLoS Computational Biology* (cover article, July 2016). This work was also presented at the 2015 annual Mount Sinai MD/PhD Retreat, as well as the *Biophysical Society Annual Meeting* and *APSA Meeting* in 2016 with competitive travel awards received for each.

Eric Sobie, PhD, Icahn School of Medicine at Mount Sinai, New York, NY

2014

Modeling Approaches to Torsades de pointes

During my summer rotation in the Sobie lab, I used computational approaches to study where forward rate dependence is possible in the context of Torsades de pointes. To do so, parameter sensitivity analyses were performed on tissue-level, human ventricular simulations. We predicted that sodium channels demonstrate forward rate dependent-like behavior, making them a potential target of interest for anti-arrhythmic drugs.

Kevin D. Costa, PhD, Icahn School of Medicine at Mount Sinai, New York, NY

2015-Present

Human Mesenchymal Stem Cell Paracrine Signaling Counteracts Heterocellular Coupling Effects on Cardiac Contractility and Arrhythmogenicity

Demonstrated benefits of human mesenchymal stem cells (hMSCs) include reduced fibrosis and enhanced contractile function, with predominant mechanisms thought to involve paracrine signaling (PS) and heterocellular coupling (HC) between hMSCs and host myocardium. In this study, we utilized mathematical modeling and three-dimensional human engineered cardiac tissues (hECTs) to test the hypothesis that hMSC-mediated PS enhances cardiac contractility and minimizes arrhythmogenicity, counterbalancing the unfavorable effects of direct HC. By incorporating such HC and PS effects into an established excitation-contraction model, simulations of hMSC PS-only and combined HC+PS effects on human cardiomyocytes nearly replicated measurements of hECT contractile function under matched experimental treatments. For example, model simulations and hECTs both demonstrated that hMSC-mediated effects were most beneficial under PS-only conditions, where developed force significantly increased compared to non-hMSC-supplemented controls. Counteracting PS and HC effects of hMSCs were also revealed in a vulnerable window (VW) analysis of tissue-level arrhythmogenicity in simulated cardiac tissue with moderate and high diffuse fibrosis; hMSC HC+PS conditions had variable effects on VW dependent on the percent of hMSCs delivered, while PS-only conditions consistently decreased the VW, thus minimizing arrhythmogenicity. Together, these findings support our hypothesis, and motivate identifying key hMSC paracrine signaling factors as an alternative cardiac therapy. This work led to a first author manuscript under submission.

Kevin D. Costa, PhD, Icahn School of Medicine at Mount Sinai, New York, NY

2015-Present

Thesis Project: The Role of Exosomes in Mesenchymal Stem Cell-Mediated Enhancement of Cardiac Electromechanics

I chose to join Dr. Costa's lab following my successful summer rotations in 2012 and 2013, and began full-time research in the summer of 2015 after completing MD1. Since that time, I have been working on the preliminary studies described in the F30 experimental training plan, and developing the structure of my doctoral thesis. I passed my thesis proposal on December 10th, 2015. Since the original F30 submission, I generated preliminary data motivating our hypothesis that exosomal-miRNA-21 is essential for human mesenchymal stem cell-paracrine mediated enhancement of cardiac contractility. Using human engineered cardiac tissues, we generated preliminary data that shows: 1) exosomes are necessary and sufficient for human mesenchymal stem cell-paracrine mediated enhancement of hECT developed force; 2) hMSC exosomal factors enhance hECT sarcoplasmic reticulum Ca²⁺ ATPase, L-type calcium channel, and anti-apoptotic gene expression; and 3) miRNA-21 is a lead factor of exosomal cargo responsible for such effects based on qPCR and our partial least squares regression mathematical model. Future work includes identifying the role of miRNA-21 in exosome mediated enhancement of human engineered cardiac tissue function, and testing the translational potential of exosomes in cardiomyopathy hECT models.

Viatcheslav Gurev, PhD and J. Jeremy Rice, PhD, IBM T.J. Watson Research Center, NY

2016

in silico Modeling of Cardiac Electromechanics

During my IBM internship this summer, I integrated the Gurev et al. high resolution computational model of the deforming heart and the Burkhoff et al. pressure-volume loop model to develop improved computational efficiency models of cardiac electromechanics and hemodynamics.

TRAINING GOALS AND OBJECTIVES

With a formal engineering background, I find myself driven to become a problem-solving physician-scientist who helps to develop tools for enhancing patient outcomes, rather than being limited by treatment options currently available. My undergraduate and Master's training at Cooper Union has provided me with the preliminary skills necessary to accomplish this goal. The next crucial step is to complete my MD/PhD training, which will build my skills as a clinician, communicator, critical thinker, teacher, and mentor, while also fostering my utilization of engineering principles to solve medically related problems. This F30 fellowship and my outlined training plan will ensure that each of these skills will be maximally developed.

My specific long-term physician-scientist goal is to integrate cardiac tissue engineering and computational research approaches to the clinic to develop translational cardiac therapies. To this end, my clinical goals involve training in Cardiology. In conversations with Sinai physician-scientist faculty such as co-mentor Roger Hajjar, MD, and advisory committee member Jason Kovacic, MD, PhD, the Cardiology field is compatible with a balanced research and clinical career. Clinical interactions with the cardiovascular patient population will be personally and professionally satisfying; furthermore, developing novel cardiac therapies using rigorous bioengineering principles will fulfill my goals to ultimately translate bench work to the clinic for improved patient outcomes. The training plan outlined in this proposal directly reflects these interests, as my project integrates engineering and computational approaches to understand and improve human mesenchymal stem cell-mediated cardiac therapies. The outcomes of my studies during this fellowship will be directly applicable to my future research.

As a future physician-scientist, it is also necessary for me to improve my ability to communicate, think critically, and teach. There is a communication barrier when translating bench work to the clinic, as researchers and clinicians often do not speak the same "language." Therefore, as a future physician-scientist, it is essential for me to be capable of presenting my research and ideas, a skill that is developed throughout the MD/PhD training. Specifically, I plan on improving my communication skills by participating in local and national scientific meetings, weekly laboratory meetings, weekly journal clubs, and monthly MSTP research seminars. These opportunities will also teach critical thinking; through thoughtful interactions with other members of the scientific community during such meetings and seminars, I will learn to ask the right questions, take intellectual risks, and think both deeply and "outside the box" about a scientific question.

Teaching throughout my MD/PhD training will further improve my communication and critical thinking skills. As a current tutor and lead teaching assistant in the cardiovascular block of the medical school physiology course, I plan to hone my teaching abilities. While further improving my own communications skills as a lecturer, teaching will ultimately assist me in the clinic to interact with patients and colleagues. Furthermore, it will open opportunities to mentor students; having benefitted from excellent mentorship by a senior graduate student, I have seen at first hand how positively it can impact one's career. Inspiring students and teaching them to think critically will provide insight into how to run a successful lab in the future, or how to direct a course. Completing all the teaching and mentorship opportunities outlined in this application will be key steps in my goal of becoming a mentor, and contributing to a mentee's education and success.

Throughout my class work and lab work during this fellowship, I will also solidify engineering concepts and their applications to medicine. For example, last year in a hands-on Makers Studio course, I implemented hydraulics, engineering design, and hardware/software concepts to develop an innovative foot-manipulating, MRI compatible-device that reinvents tarsometatarsal ligament injury diagnostics for enhanced Lisfranc injury treatment and management. My thesis work also directly implements bioengineering principles, as we develop computational models and tissue engineering technologies to study the role of exosomal miRNA-21 in human mesenchymal stem cell-mediated enhancement of cardiac contractility.

Overall, the proposed F30 project will assist me in accomplishing both my short- and long-term career goals. Key steps for me to become a successful physician-scientist include refining skills in communication, critical thinking, teaching, and mentoring, while also reinforcing engineering principles for developing novel cardiac therapies. I am confident that at the Icahn School of Medicine at Mount Sinai, with a mentoring team that supports my scientific directions and career plans, I will receive an invaluable training experience that will help me fulfill my career aspirations.

ACTIVITIES PLANNED UNDER THIS AWARD

Year 1: PhD phase

Activity	Description	Time
Research	Having passed my Thesis Proposal, most of my time will be spent on laboratory work to complete the Aims described in the Research Strategy. I will also spend time on manuscript submission and conference presentations. I will defend my dissertation in Year 2.	80%
Coursework	With required coursework completed, I will take selected advanced electives at CCNY (e.g., <i>BME 2000: Cell and Tissue Engineering</i>).	5%
Teaching	To increase my lecturing skills, as well as my understanding of the human body, I will continue working in the medical school as the physiology cardiovascular block lead Teaching Assistant; I will also be a Teaching Assistant in <i>BSR1802: Quantitative Physiology</i>	5%
Clinical	To maintain clinical acumen during my PhD phase, I will continue to attend Dr. Raj Sahulee's rounds in pediatric cardiology. I will also participate in the MSTP clinical refreshers offered by Dr. Talia Schwartz, Associate Director of the MSTP program.	5%
Miscellaneous	I will attend grand rounds in the Cardiovascular Institute, as well as weekly seminars in the Cardiovascular Research Center and the Biophysics and Systems Pharmacology multi-disciplinary training area to keep up with advancing research. I will also attend at least one relevant national scientific conference (e.g., AHA, BPS, BMES) and monthly Medical Scientist Ground Rounds intended for MSTP students.	5%

Year 2: PhD phase

Activity	Description	Time
Research	Most of my time will be spent on laboratory work to complete the Aims described in the Research Strategy and to defend my dissertation.	80%
Coursework	With required coursework completed, I will take selected advanced electives at CCNY (e.g., <i>CSc 44200: Systems Simulation</i>). The <i>Responsible Conduct of Research</i> course will be repeated at least once every four years.	5%
Teaching	I will continue working in the medical school as the physiology cardiovascular block lead Teaching Assistant.	5%
Clinical	I will continue my clinical experiences, as outlined in Year 1. I will also participate in the MSTP clinical refreshers offered by Dr. Talia Schwartz, Associate Director of the MSTP program.	5%
Miscellaneous	I will continue attending grand rounds, works in progress, seminars, and conferences as outlined in Year 1.	5%

Year 3: Pre-clinical phase MD2

Activity	Description	Time
Research	Finalizing manuscript revisions and resubmission as needed.	5%
Coursework	I will spend the majority of time taking required 2 nd year medical school courses. The <i>Responsible Conduct of Research</i> course will be repeated at each stage of my career.	50%
Teaching	I will continue my teaching responsibilities, as outlined in Year 1.	5%
Clinical	I will also spend Wednesdays in the clinic taking histories and performing physical exams during the <i>Art and Science of Medicine</i> course.	10%
Miscellaneous	I will prepare for the Step 1 USMLE exam	30%

Year 4: Clinical phase MD3

Activity	Description	Time
Research	My time will be devoted to clinical rotations.	0%
Coursework	My time will be devoted to clinical rotations.	0%
Teaching	My time will be devoted to clinical rotations.	0%
Clinical	I will complete 3 rd year clinical rotations in various specialties.	100%
Miscellaneous	My time will be devoted to clinical rotations.	0%

SPECIFIC AIMS

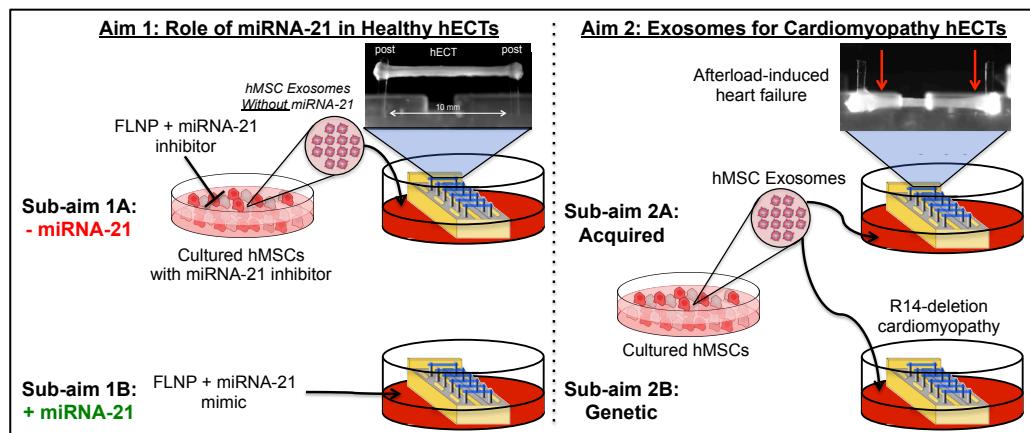
An emerging approach to treat patients with heart failure from non-ischemic dilated cardiomyopathy (NIDCM) involves delivery of cells to repair failing myocardium. NIDCM clinical trials document promising benefits of human mesenchymal stem cells (hMSCs), underscoring a need to better understand and exploit mechanisms governing interactions between hMSCs and myocardium. hMSC paracrine functional benefits predominately involve enhanced angiogenesis, reduced fibrosis, and immunomodulation. Moreover, hMSC paracrine factors have been shown to inotropically impact contractility by altering cardiomyocyte ion channel/pump activity via PI3K/Akt signaling. However, studies fail to identify the key inotropic components of the hMSC secretome, providing motivation for our proposal. *We propose utilizing human engineered cardiac tissues (hECTs) as an in vitro model of healthy and diseased myocardium to identify the key inotropic agents of the hMSC secretome.*

Consistent with our mathematical models, our lab recently discovered that hMSCs enhance hECT contractile force predominantly through paracrine signaling, counteracting the adverse risks of direct hMSC-hCM heterocellular coupling. Importantly, we discovered that the exosomal component of the hMSC secretome is necessary and sufficient for hMSC-paracrine mediated enhancement of hECT contractility. This is consistent at the molecular level, where mRNA expression of sarcoplasmic/endoplasmic reticulum calcium transport ATPase, L-type calcium channel, and anti-apoptotic factors is significantly increased for hECTs treated with the total and exosome-only fractions of the hMSC secretome, whereas hECTs treated with the exosome-depleted fraction of the hMSC secretome are unchanged relative to control. Furthermore, by utilizing a systems biology approach to form relationships between hECT contractile function outputs with input exosomal miRNA data, we predicted exosomal miRNA-21 as a lead candidate responsible for the favorable contractility effects of hMSC paracrine signaling. We later demonstrated via qPCR that miRNA-21 levels are significantly increased in hECTs supplemented with hMSC exosomes and hMSC total conditioned media relative to control conditions, motivating our central hypothesis that *exosomal miRNA-21 is essential for hMSC paracrine-mediated enhancement of contractile performance*. This hypothesis will be tested in two Aims:

Aim 1: To identify the role of exosomal miRNA-21 in hMSC paracrine mediated enhancement of healthy hECT contractility. We will investigate whether exosomal miRNA-21 is necessary and sufficient for hMSC paracrine mediated enhancement of hECT contractility. **Sub-aim 1A:** To determine whether exosomal miRNA-21 is necessary for hMSC paracrine enhancement of healthy hECT contractility via delivery of exosomes derived from miRNA-21 inhibited hMSCs. **Sub-aim 1B:** To test whether exosomal miRNA-21 is sufficient for hMSC paracrine mediated enhancement of healthy hECT contractility by miRNA-21 mimic delivery into healthy hECTs. Both Sub-aims will utilize an in house formulated lipidoid nanoparticle (FLNP) for delivery of miRNA-21 inhibitor and mimic. Treatment potency will be assessed via hECT contractile function, gene expression, and structural analysis.

Aim 2: To test the inotropic potency of hMSC exosomes in the context of NIDCM.

hECT models of acquired and genetic non-ischemic heart failure will be used as translational contractility assays to assess the inotropic potency of hMSC exosomes. **Sub-aim 2A:** To test the role of hMSC exosomes on recovery of contractility using an afterload-induced acquired heart failure hECT model. **Sub-aim 2B:** To test the role of hMSC exosomes on recovery of contractility in our patient-derived phospholamban mutant (R14del) hECT model of genetic NIDCM.



Overall Significance: This proposal uses state-of-the-art tissue engineering to identify the role of exosomes and their essential cargo in hMSC-mediated inotropic enhancement of cardiac contractility to assess their cardiotherapeutic potential. This dissertation project directly tackles an NIH/NHLBI topic of special interest, as we will look at the role of exosomes as paracrine signaling mediators in cardiovascular disease. Additionally, the project will provide the candidate with rigorous multi-disciplinary training in tissue engineering, systems biology, electrophysiology, human stem cell biology, and biochemistry, in preparation for a career as a physician scientist focused on developing innovative cardiotherapies.

RESEARCH STRATEGY

Background and Significance

Non-ischemic cardiomyopathy: Heart failure (HF) remains a leading cause of morbidity and mortality in Western countries, with a prevalence of approximately 5.4 million people in the United States alone.¹ Despite advancements in the field, HF still has a poor prognosis, as approximately 50% of patients die within 5 years.¹ Non-ischemic dilated cardiomyopathy (NIDCM)—ventricular dilation and systolic dysfunction in the absence of coronary artery disease—is a subset of HF impacting 1 in 20,000 individuals per year,¹ is the most common form of pediatric cardiomyopathy,² and is the leading diagnosis (54%) among heart transplant recipients.³ These statistics highlight a need to develop therapeutic strategies for restoring cardiac performance in NIDCM.

Mesenchymal stem cell therapy for NIDCM: Bone marrow-derived human mesenchymal stem cells (hMSCs) are an emerging approach to treat HF.⁴⁻⁷ However, much less is known about the benefits of hMSC therapy in the context of NIDCM. In one case study, intracoronary administration of autologous hMSCs to an 11-year-old boy with NIDCM and class IV HF led to an increase of left ventricular ejection fraction from 20% to 42%.⁸ In a recent clinical trial, hMSC injection into NIDCM patients increased ejection fraction with improvements in safety and efficacy endpoints.⁹ Thus, hMSC therapy for NIDCM is an important area for investigation.

Mechanisms of hMSC therapy: Predominately through paracrine signaling mechanisms (via soluble factors and bilayer membrane-bound nanovesicles called exosomes)¹⁰⁻¹², transplantation of hMSCs has been shown to be immunomodulatory^{7, 13}, enhance angiogenesis¹⁴⁻¹⁶, and decrease fibrosis.^{10, 16} Moreover, hMSC paracrine factors have been shown to increase excitation-contraction coupling by increasing L-type calcium channel and sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a) activity via the phosphatase and tensin homologue/phosphoinositide 3-kinase/protein kinase B (PTEN/PI3K/Akt) signaling pathway¹⁷, a pathway known to be modulated by miRNA-21.^{18, 19}

miRNA-21 in cardiomyocyte function: Based on our preliminary work and current literature, we hypothesize that exosomal miRNA-21 is responsible for hMSC paracrine mediated enhancement of hECT contractility. miRNA-21 is the only miRNA that is consistently in the top 5 for most abundantly found in hMSC exosomes across three independent studies.²⁰⁻²² Interestingly, cardiac progenitor cell exosomal miRNA-21 has been shown to prevent cardiomyocyte apoptosis by targeting programmed cell death 4 (PDCD4), known to be downstream of the PTEN/PI3K/Akt signaling pathway.²³ This is consistent with the findings by Wei et al.²⁴ and Cheng et al.²⁵, where miRNA-21 also regulates cardiomyocyte apoptosis by targeting PDCD4. The PTEN/PI3K/Akt signaling pathway has been shown to increase L-type calcium channel (LTCC) activity, consistent with findings from DeSantiago et al.^{26, 27} miRNA-21 has also been shown to bind to phospholamban strongly²⁸ or activate endothelial nitric oxide synthase²⁹, both of which increase SERCA2a activity.^{17, 28}

Human engineered cardiac tissues: By combining tissue-engineering technology with human stem cell biology, a major breakthrough has been the development of in vitro models of functional human myocardium. In our lab, human engineered cardiac tissues (hECTs), created with human pluripotent stem cell-derived cardiomyocytes, are used as an in vitro assay to evaluate contractile performance (Fig. 1A-B) by monitoring developed force (DF) and maximum rates of contraction (+dF/dt) and relaxation (-dF/dt).^{30, 31} Our hECTs recapitulate key aspects of cardiac muscle physiology including excitation-contraction coupling, the Frank-Starling mechanism, and pharmacologic responses to inotropic agents such as Milrinone and Dobutamine (Fig. 1C-D). Our lab is also capable of modeling characteristics of NIDCM by creating hECTs with induced pluripotent stem cells from a patient harboring the R14del mutation in the phospholamban gene (Fig. 1E-F)^{32, 33}, demonstrating our ability to recapitulate key elements of NIDCM phenotype.

Significance of proposal: Overall, the proposal significance includes: 1) exploring new cardiac therapy strategies for NIDCM patients; 2) using human cells in healthy and diseased cardiac tissue engineering models to harness the hMSC secretome for translational potential; 3) addressing an NHLBI topic of special interest, as we study the role of exosomes as paracrine signaling mediators in cardiovascular disease; and 4) providing the candidate with rigorous multi-disciplinary training in tissue engineering, systems biology, stem cell biology, electrophysiology, and biochemistry, with a focus on developing innovative stem cell-based cardiac therapies.

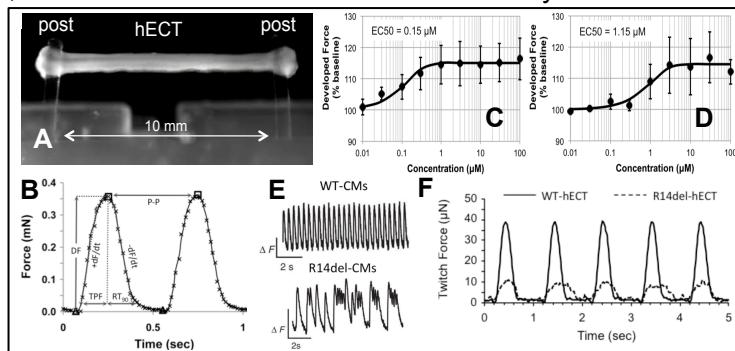


Figure 1. hECT contractility assay. A) hECTs created in our force-sensing mold. B) Example twitch force at 2 Hz pacing. Pharmacological responses to C) milrinone and D) dobutamine inotropic agents. E) Example calcium transients of wild type (WT)- and R14del- hiPSC-cardiomyocytes. F) Example twitch tracings from WT and R14del hECTs at 1 Hz pacing.

Approach

Preliminary Studies:

hMSC paracrine signaling (PS) enhances hECT contractility, counteracting unfavorable heterocellular coupling (HC): Our multi-tissue bioreactor system³¹ (**Fig. 2A-C**) was designed for isolating hMSC HC and PS interventions via simultaneous culture of six hECTs comprised of either unsupplemented (-hMSC) or 10% hMSC-supplemented (+hMSC) cellular composition, allowing four different experimental groups (**Fig. 2D**): 1) -hMSC hECTs only (black); 2) +hMSC hECTs only (purple); or 3-4) alternating +hMSC and -hMSC hECTs in a shared paracrine media bath (red and blue, respectively).

In parallel, we developed novel mathematical models of both the HC (published cover article in *PLoS Comput Biol*)³⁴ and PS (manuscript submitted) effects of hMSCs on cardiomyocyte electrophysiology and excitation-contraction. To compare our model and hECT DF under matched conditions, we assumed the above four hECT groups correspond to approximately: 1) 0% HC + 0% PS; 2) 10% HC + 10% PS; 3) 10% HC + 5% PS; and 4) 0% HC + 5% PS hMSC treatment interventions, respectively (**Fig. 2E**), in our excitation-contraction mathematical model.

Excitation-contraction simulations of PS-only (0% HC + 5% PS) and combined HC+PS (10% HC + 5% PS and 10% HC + 10% PS) effects of hMSCs on human stem cell-derived cardiomyocytes replicated measurements of hECT contractile function at day 7 under matched experimental hMSC-mediated treatments (**Fig. 2E**). All twelve log-normally distributed trial simulations were within one standard deviation of each experimental group for DF. Both model simulations and hECTs demonstrated that the hMSC-mediated effects were most beneficial under PS-only conditions (0% HC + 5% PS), where developed force significantly increased by approximately 3.5-fold compared to non-hMSC-supplemented controls during physiologic 1-Hz pacing. Together, these findings suggest *hMSC PS counteracts HC and enhances hECT contractility, providing a scientific premise to isolate the paracrine mechanism and identify key inotropic hMSC PS factors*.

hMSC exosomes are taken up by fibroblasts and cardiomyocytes: To support our hypothesis that hMSC exosomes are the key component of the hMSC secretome for paracrine mediated enhancement of hECT contractility, we first confirmed that Calcein-stained hMSC exosomes (green)—isolated after five days of hMSC culture in serum free defined media (SFDM; RPMI media + B27 supplement with insulin) by established protocols³⁵ and confirmed via dynamic light scattering (**Fig. 3A**)—are taken up by Hoescht stained (blue), fluorescence activated cell sorted, human stem cell-derived SIRPa+ cardiomyocytes (**Fig. 3B**) and CD90+ fibroblasts (**Fig. 3C**) within 48 hours of treatment.

hMSC exosomes are necessary and sufficient for hMSC paracrine mediated enhancement of hECT function: We tested the hypothesis that hMSC exosomes are the key component of the hMSC secretome for paracrine mediated enhancement of hECT contractility by replacing hECT SFDM media with the following treatments following baseline contractile function testing on day 5: 1) SFDM (Control); 2) hMSC conditioned media (hMSC Cdm); 3) SFDM supplemented with hMSC exosomes (hMSC exo); or 4) hMSC exosome-depleted conditioned media (hMSC exo-depl). hECTs were cultured an additional 5 days, and then DF was measured again. As shown in **Fig. 4**, the hMSC Cdm and hMSC exo treatments led to statistically significant increases in DF, whereas the hMSC exo-depl group was unchanged relative to control. *These preliminary findings demonstrate the presence of inotropically cardioactive exosomes secreted by hMSCs that significantly increase hECT twitch force, with negligible inotropic contribution from hMSC-secreted soluble factors.*

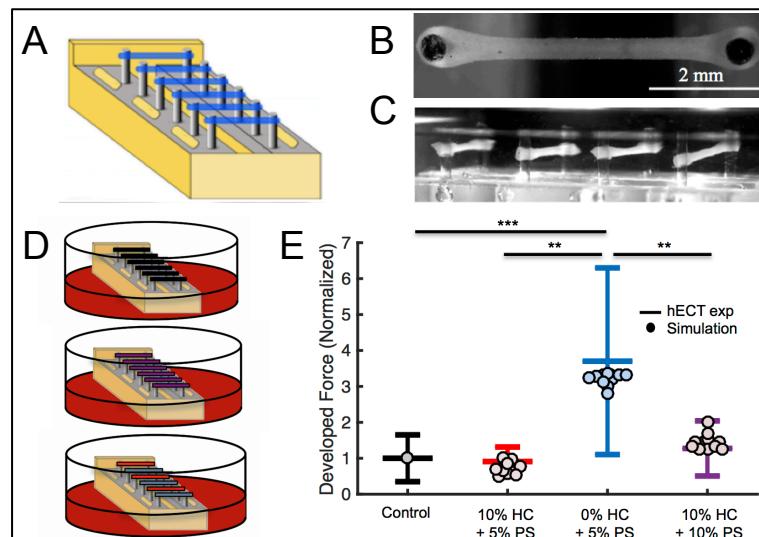


Figure 2. hMSC PS counteracts HC and enhances hECT contractility. A) Schematic of bioreactor; B) top view of hECT; C) oblique view of hECTs; D) Schematic of the experimental groups tested; E) Comparison of control, 10% HC + 5% PS, 0% HC + 5% PS, and 10% HC + 10% PS hECT measurements (mean +/- SD, n = 9-18) to twelve log-normally distributed simulations (circles) for DF (legend inset, with hECT exp representing empirical hECT data. All data are normalized to control. * p < 0.01; ** p < 0.001; *** p < 0.0001 based on hECT experiments. Mayourian et al. (*submitted*)

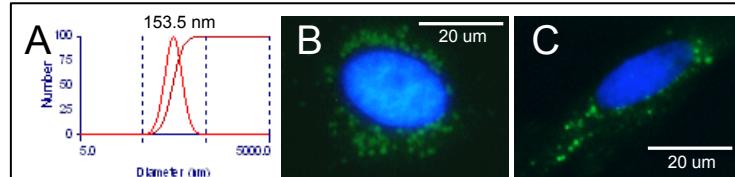


Figure 3. Cardiomyocyte and fibroblast uptake of hMSC exosomes. A) Dynamic light scattering characterizes hMSC exosomes with a diameter of 153.5 nm. Calcein-stained hMSC exosomes (green) are taken up by Hoescht stained (blue), fluorescence activated cell sorted, human embryonic stem cell-derived SIRPa+ cardiomyocytes (B) and CD90+ fibroblasts (C) within 48 hours of treatment.

These findings on the functional role of hMSC exosomes are corroborated by molecular analysis. Following five days of treatment as above, hECTs were snap-frozen for prospective real time quantitative polymerase chain reaction (qPCR) of cardiac-specific (α -MHC, β -MHC)³⁰, apoptosis (CASP3, CASP9, BAX, BCL2), and calcium handling (SERCA2a, LTCC)³⁶ genes, using β 2-microglobulin housekeeping gene. mRNA levels of SERCA2a and LTCC significantly increased for hECTs treated with hMSC CdM and hMSC exo (Fig. 4B), while they significantly decreased for the BAX/BCL2 ratio (Fig. 4C), an established apoptosis marker.^{37, 38} Together, these findings demonstrate at functional and molecular levels that hMSC exosomes are necessary and sufficient for hMSC paracrine mediated enhancement of hECT function, motivating investigation of the key exosomal cargo responsible for these cardioactive effects.

Exosomal miRNA-21 is a lead candidate responsible for hMSC paracrine mediated

enhancement of hECT contractility: Recently, it was reported that proteins are less abundant in exosomes than the exosome-depleted fraction of the hMSC secretome,²¹ which we found to have no effect on hECT function (Fig. 4A). Furthermore, extensive literature suggests exosomal miRNAs constitute a significant functional component of exosomes.³⁹⁻⁴³ Therefore, to identify lead miRNA candidates responsible for cardioactive hMSC exosome effects, we integrated a systems biology approach as described elsewhere;^{44, 45} specifically, we supplemented hECTs with SFDM, and with CdM from human foreskin fibroblasts (HFFs), human adult cardiac fibroblasts (hACFs), and hMSCs, each known to have their own miRNA signatures.^{22, 46, 47} (HFF and hACF CdM effects on hECTs not shown). We then collected HFF, ACF, and hMSC exosome miRNA microarray and RNA-seq data from published studies⁴⁶, Gene Expression Omnibus (GEO) accession GSE71241,⁴⁷ and GEO accession GSE76175²² respectively, and performed an unbiased partial least squares regression (PLSR) analysis between input miRNA content from exosomes of each treatment condition and output hECT contractile function and mRNA expression data. Together, this model comprised of the top 20 miRNAs found in hMSC exosomes with established cardiac activity, as well as the responses of hECT DF and mRNA expression of LTCC, SERCA2a, and BAX/BCL2. The PLSR model trained with a predictability of 98.3%. The loading plot (Fig. 5A) demonstrates that miRNA-21 and miRNA-22 clustered with DF, making them our theoretical lead miRNA candidates. To test this, we performed qPCR of SFDM, hMSC CdM, hMSC exo, and hMSC exo-depl treated-hECTs for levels of miRNA-21, miRNA-22, and miRNA-486 (control), normalized to SNORD44, an established housekeeping gene.⁴⁸ Remarkably, treating hECTs with hMSC CdM and hMSC exo led to significant increases in miRNA-21 levels in hECTs (Fig. 5B). These preliminary findings together with previous literature motivate our hypothesis that exosomal miRNA-21 has an essential role in hMSC paracrine mediated enhancement of hECT contractile function.

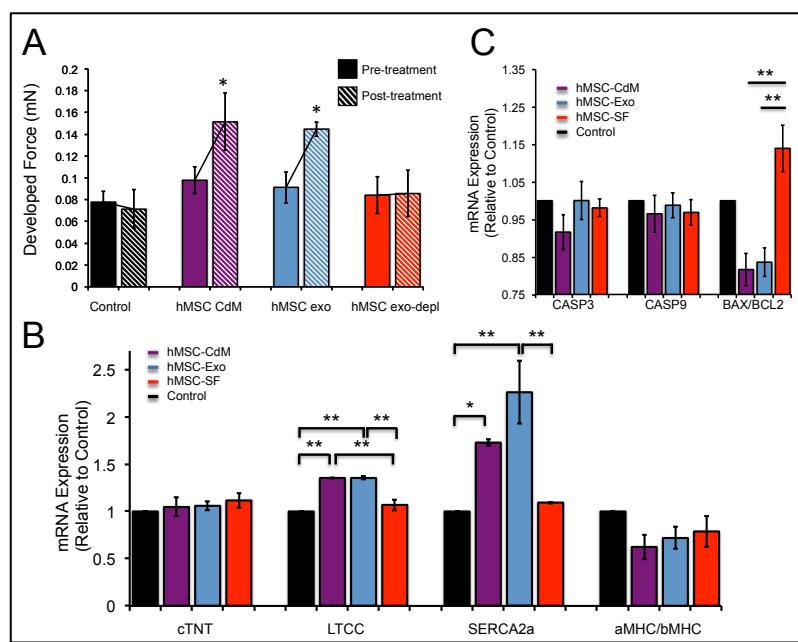


Figure 4. Effects of hMSC exosomes on hECT function and mRNA levels. A) Exosome contractility assay shows hECT DF during 0.5-Hz pacing (mean \pm SEM, n=4-7) at pre-treatment (day 5) and 5-days post-treatment using: 1) SFDM; 2) hMSC CdM; 3) hMSC exo; and 4) hMSC exo-depl. * p < 0.05; p-values from paired t-tests. hECTs from each group (n = 3) were snap-frozen for qPCR on day 10, where B) cardiac-specific and C) apoptotic genes were studied. * p < 0.05, ** p < 0.01; p-values from ANOVA with post-hoc Tukey test.

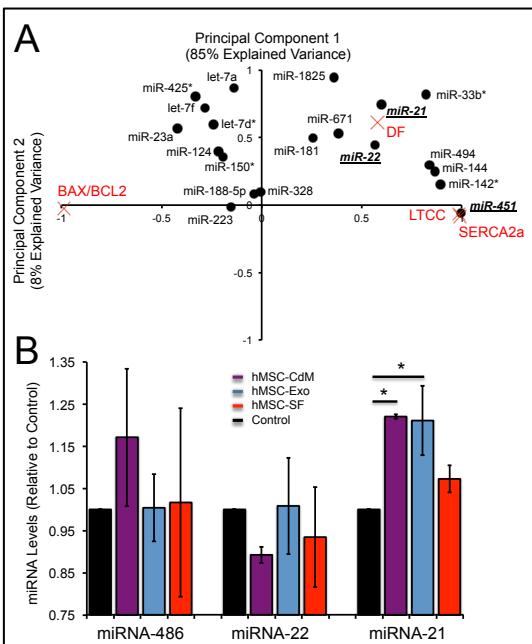


Figure 5. Prediction and experimental testing of miRNA-21 as a lead candidate. A) PLSR loading plot shows response variables (e.g. DF, LTCC, SERCA2a, BAX/BCL2; labeled in red) covarying with miRNA. Top three miRNA candidates (i.e., miRNA-21, miRNA-22, and miRNA-451) correlating with response variables are underlined. B) qPCR of SFDM, hMSC CdM, hMSC exo, and hMSC exo-depl treated-hECTs for levels of miRNA-486 (control), miRNA-22, miRNA-21 (mean \pm SEM, n=4). * p < 0.05; p-values from post-hoc Tukey ANOVA test.

Aim 1: To identify the role of exosomal miRNA-21 in hMSC-mediated enhancement of hECT contractility.

Rationale: hMSC delivery is an emerging cell-based therapeutic for NIDCM, underscoring a need to better understand and exploit the underlying mechanisms governing interactions between hMSCs and myocardium. hMSC paracrine factors have been shown to inotropically impact contractility; however, the key ionotropic constituents of the hMSC secretome remain unresolved. Our new preliminary data demonstrate that the exosomal component of the hMSC secretome is necessary and sufficient for hMSC-paracrine mediated enhancement of hECT contractility. Furthermore, by utilizing an integrated systems biology approach, we predicted exosomal miRNA-21 as a lead candidate responsible for the favorable contractility effects of hMSC paracrine factors later found to be significantly increased in hMSC exosome-supplemented hECTs relative to control. miRNA-21 is the only miRNA that consistently ranks among the top 5 most abundantly found in hMSC exosomes across three independent studies.²⁰⁻²² Furthermore, wide-ranging literature suggests miRNA-21 is highly involved in the cardiomyocyte PTEN/PI3K/Akt signaling pathway,²³⁻²⁵ which Desantiago et al.¹⁷ and others^{49, 50} showed to be the dominant pathway regulating hMSC paracrine mediated cardiac inotropic effects.

Experimental Design:

Sub-aim 1A: To test the hypothesis that exosomal miRNA-21 is necessary for hMSC paracrine mediated enhancement of healthy hECT contractility. To address this Sub-aim, hECTs will be created in our custom multi-tissue bioreactor system using a modified version of our published methods.³⁰ A directed differentiation protocol described elsewhere⁵¹ will be used to derive hCMs from H7 human embryonic stem cells (NIH Registration Number 0061). Following the 20-day differentiation, cells are passaged and added to 2 mg/mL type-I bovine collagen (Life Technologies) and 0.9 mg/mL Matrigel (Corning) for a final concentration of 20 million cells/mL, or 500,000 cells per tissue. The multi-hECT bioreactor, with 6 pairs of PDMS force-sensing posts, is then inverted and placed into a baseplate containing 6 channels, each filled with 25 μ L of the above cell-matrix solution. After two hours at 37°C and 5% CO₂, DMEM media (Sigma-Aldrich) with 10% neonatal bovine serum, 1% penicillin-streptomycin, and 0.2% amphotericin B (i.e., NBS media) is added to cover the hECTs. After 48 hours the baseplate is removed, resulting in six hECTs each 6 mm long, suspended between two PDMS posts (**Fig. 2**). NBS media is then replaced with SFDM, composed of RPMI 1640 medium with B27 supplement (Life Technologies), 1% penicillin-streptomycin, and 0.2% amphotericin B under normoxic (20% O₂) conditions. The tissues self-assemble and begin spontaneous beating within 2-3 days. For quality assurance of our differentiations, live cell sorting will be used each month to isolate SIRP α ⁺ cardiomyocytes⁵² and CD90⁺ stromal cells⁵³⁻⁵⁵ to confirm a differentiation efficiency of approximately 80%.

Five days after tissue formation, hECT contractile testing will be performed for DF, \pm dF/dt, and force-frequency relationship using methods previously described (**Figs. 1-2**).³⁰ After functional testing, hECTs will be treated in SFDM with either: 1) exosomes derived from hMSCs inhibited of commercial miRNA-negative control; or 2) exosomes derived from hMSCs inhibited of miRNA-21. To inhibit hMSCs of miRNA-21 or miRNA-negative control, we will deliver formulated lipidoid nanoparticles (FLNP) recently developed in our lab⁵⁶ carrying enhanced green fluorescence protein (eGFP) mRNA and the miRNA inhibitor (miRNAl) of interest (ThermoFisher Scientific), creating a cardiotropic FLNP-eGFP-miRNAl complex. Normoxic cultured hMSCs will be transfected with the FLNP-eGFP-miRNAl complex for 24 hours. Following successful transfection, hMSCs will be cultured with SFDM under normoxic conditions. After five days of serum-free culture, hMSC exosomes inhibited of miRNA-21 or miRNA-negative control will be isolated using an established protocol³⁵ (**Fig. 2-3**). These exosomes will then be delivered to hECTs in SFDM as described above.

Five and ten days post-treatment, hECTs will be reassessed for contractile function. On day 15, hECTs will be snap-frozen and randomly assigned for prospective testing by: 1) immunohistochemistry (IHC) and electron microscopy for quantitative analysis of microstructural organization (e.g. sarcomeres, gap junctions, T-tubules) as we previously described;^{30, 57} or 2) qPCR of cardiac-specific (e.g. α -MHC, β -MHC)³⁰, apoptosis (e.g. CASP3, CASP9, BAX, BCL2), and calcium handling (SERCA, LTCC)³⁶ genes.

Sub-aim 1B: To test the hypothesis that exosomal miRNA-21 is sufficient for hMSC paracrine mediated enhancement of healthy hECT contractility. To address this Sub-aim, hECTs will be constructed using the methods previously described. Five days after tissue formation, hECT contractile testing will be performed for DF, maximum \pm dF/dt, and force-frequency relationship using methods previously described (**Figs. 1-2**).³⁰ After functional testing, hECTs will be treated with SFDM supplemented with either: 1) FLNPs carrying miRNA-negative control mimics (ThermoFisher Scientific); or 2) FLNPs carrying miRNA-21 mimics (ThermoFisher).

To deliver miRNA mimics (miRNAm), we will also utilize FLNP carrying eGFP and the miRNAm. Specifically, hECTs will be transfected with the FLNP-eGFP-miRNAm complex added to SFDM on day 5 post-

functional testing. Following 24 hours of transfection, hECTs will continue to be cultured with SFDM under normoxic conditions. Five and ten days post-treatment, hECTs will be reassessed for contractile function. On day 15, hECTs will be snap-frozen and randomly assigned for either prospective IHC, electron microscopy, or qPCR as described in Sub-aim 1A.

Samples and timeline: For each Sub-aim, there are two conditions, yielding a total of four experimental groups. Based on our previous experience of an 80% success rate in making hECTs, a total of 50 hECTs will be created in this Aim to ensure a sample size of 10 hECTs per group, translating to a realistic 12 months to accomplish. This is sufficient to detect a 50% change in DF with $\alpha=0.05$ and $\beta=0.2$ based on preliminary data.

Anticipated Results: In Sub-aim 1A, we expect transfection of hMSCs with FLNP-eGFP-miRNAi complex will lead to exosomes without miRNA-21, leading to a loss of hECT contractility enhancement not seen with controls. This will imply that miRNA-21 is necessary for exosomal-mediated enhancement of hECT contractile function. On the other hand, in Sub-aim 1B, we expect transfection of hMSCs with FLNP-eGFP-miRNAm complex will lead to increased miRNA-21 levels in hECTs, leading to a gain of hECT contractility enhancement not seen in controls, and comparable to the total hMSC exosome treatment condition (Fig. 3). This will imply that miRNA-21 is sufficient for exosomal-mediated enhancement of hECT contractile function. Together with consistent findings at the structural and molecular levels, this aim would demonstrate that exosomal miRNA-21 is necessary and sufficient for hMSC paracrine mediated enhancement of hECT contractility.

Potential Pitfalls and Alternative Strategies: In our lab, FLNP delivery has been successful to HeLa and hESC cells in vitro, as well as to rat and pig myocardium in vivo by intramyocardial and intracoronary delivery.⁵⁶ Therefore, this is a promising approach for miRNAi and miRNAm delivery to hMSC cultures and hECTs, respectively. However, in the unexpected case of limited delivery efficiency, we will switch to a lipofectamine delivery method of miRNAi and miRNAm.^{58,59} While miRNA-21 appears to be a promising lead candidate responsible for hMSC paracrine mediated enhancement of hECT contractility, it is possible that this miRNA is not necessary and/or sufficient. Such an outcome would lead us to: 1) test other lead candidates from our partial least squares regression model (e.g., miRNA-451); and 2) perform RNA-seq on hMSC exosomes, as well as hECTs treated with serum-free media (control), hMSC conditioned media, hMSC exosomes only, and exosome-depleted hMSC conditioned media. This would provide insight into the possible exosomal cargo and mechanisms of exosome mediated enhancement of cardiac contractility. We acknowledge that while miRNA-21 has beneficial effects on cardiomyocytes, it has been reported that selectively increasing miRNA-21 in fibroblasts augments ERK-MAP kinase activity, leading to an increase in cardiac fibroblast survival, fibrosis, remodeling, and cardiac dysfunction.⁶⁰ The high percentage of cardiomyocytes and low percentage of fibroblasts in our healthy hECTs may conceal such adverse effects. In the context of future cardiotherapeutics, we would propose developing and delivering cardiomyocyte-targeting nanoparticles⁶¹⁻⁶⁴ carrying miRNA-21 mimics to circumvent such potential adverse effects.

Aim 2: To test the inotropic potency of hMSC exosomes in the context of NIDCM.

Rationale: This aim is designed to assess the efficacy of delivering hMSC exosomes in the context of HF from NIDCM. Inotropic therapy is a standard but often inadequate form of medical management of NIDCM. Bone marrow-derived human mesenchymal stem cells (hMSCs) are an emerging approach to treat HF.⁴⁻⁷ However, much less is known about the benefits of hMSC therapy in the context of NIDCM. Importantly, we discovered that the exosomal component of the hMSC secretome is necessary and sufficient for hMSC-paracrine mediated enhancement of healthy hECT contractility, as well as calcium handling and anti-apoptotic gene expression. We will examine the efficacy of hMSC exosomes using two hECT-based in vitro models of non-ischemic HF: 1) an acquired overload-induced hypertrophic cardiomyopathy;⁶⁵ and 2) a genetic cardiomyopathy model based on the PLN-R14del genetic mutation associated with a familial dilated cardiomyopathy.^{66, 67} The ultimate goal of this research is to identify hMSC-mediated paracrine signaling factors that directly enhance cardiomyocyte contractility in order to capture the benefits of hMSC therapy while circumventing potential risks of direct heterocellular coupling for treating HF.

Experimental Design:

Sub-aim 2A: To test the role of hMSC exosomes on recovery of contractility using an afterload-induced acquired heart failure hECT model: As described above, hECTs will be created and cultured for 5 days to allow

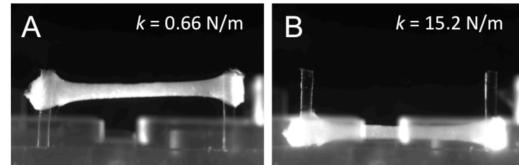


Figure 6. Protocol for afterload-induced HF. Lowering the height of the hECT on posts from A) to B) increases the effective spring constant over 20-fold increasing resistance on the contracting tissue.

proper tissue compaction and stabilization of coordinated spontaneous beating. On day 5, baseline contractile function will be measured; following contractile testing, hECTs will be lowered to the base of the posts (**Fig. 6**) and returned to the incubator for an additional 5 days. Such intervention increases the effective post stiffness over 20-fold, thereby increasing mechanical afterload and minimizing systolic tissue shortening. This will induce a pathological hypertrophic response similar to the rat engineered cardiac tissue model of hypertrophic cardiomyopathy described by Hirt et al.⁶⁵

Three experimental groups will be randomly assigned on day 5 prior to initial testing: 1) Sham, where the hECT will be moved to the bottom of the posts on day 5, and then immediately move back to top of posts for the remainder of experiment with no hMSC exosome treatment; 2) Treated overload, where the hECT will be moved to the bottom of the posts on day five and the remainder of the experiment, with hMSC exosome treatment added on day 5 after testing; and 3) Untreated overload, where the hECT will be moved to the bottom of the posts on day five and the remainder of the experiment without any treatment. Contractile function will subsequently be assessed on day 10 by temporarily moving the hECT back up to the top of the posts to evaluate the effects of mechanical overload. The treated overload experimental hECT group will again be treated with hMSC exosomes on day 10. Finally, after 5 more days of treatment (i.e., day 15) the contractile function will be tested again, and hECTs will subsequently be snap-frozen and randomly distributed for prospective IHC, electron microscopy, and qPCR as described in Sub-aim 1A.

Sub-aim 2B: Testing the role of hMSC exosomes on recovery of contractility for genetic cardiomyopathy: Using our extensively characterized patient-specific induced pluripotent stem cell PLN-R14del cell lines,³² we will create R14del-hECTs that exhibit key characteristics of the familial dilated cardiomyopathy phenotype, as shown in **Fig. 1E-F** and elsewhere.³³ Using a protocol analogous to Sub-aim 2A, the R14del-hECTs will be cultured until day 10 to acquire the DCM phenotype without any treatment. Following baseline contractile function testing at day 10, R14del-hECTs will be split into two groups: 1) hMSC exosome treatment; and 2) no treatment (i.e., SFDM control). R14del-hECTs will then be cultured for 5 more days with functional measurements at day 15. R14del-hECTs will subsequently be snap-frozen and randomly assigned for prospective IHC, electron microscopy, and qPCR as described in Sub-aim 1A.

Samples and timeline: In Sub-aims 2A and 2B, we have a repeated measures study design. Therefore, a sample size of 8-10 hECTs per groups should be sufficient to detect functional differences with each group, corresponding to a realistic 6-12 months to accomplish.

Anticipated Results: In Aim 2, it is anticipated that hMSC exosomes will have a positive effect on the contractile performance of hECT models of genetic and afterload-induced non-ischemic HF. In Sub-aim 2A, we expect the Untreated overload group to have decreased function relative to Sham. On the other hand, we anticipate the Treated overload group to increase function relative to the Untreated overload group, with comparable function to the Sham group. In Sub-aim 2B, we anticipate the hMSC exosome treatment group to have greater contractility than the untreated group. In both Sub-aims, we expect gene expression analyses to be consistent with the functional outputs. Together, these findings would demonstrate that hMSC exosomes are capable of enhancing contractility not only in healthy hECTs, but also in hECT models of non-ischemic cardiomyopathy.

Potential Pitfalls and Alternative Strategies: While hECTs provide a more biomimetic 3D environment than standard in vitro cell cultures and allow direct measurement of muscle contractile properties, we are aware that hECTs remain a simplified surrogate for native adult cardiac muscle.³⁰ To this end, it is possible our preliminary findings that hMSC exosomes enhance healthy hECT contractility may not translate to the animal models (beyond the scope of this proposal) to be tested as part of my Sponsor's R01 proposal. Nevertheless, this outcome would provide scientific value by contributing useful insight into the underlying reasons for modest improvements of hMSC therapy found clinically compared to in vitro studies. As the field progresses in developing strategies for driving maturation of human pluripotent stem cell derived-cardiomyocytes, the hECT model will improve while still being amenable to adding specific cell populations, biochemical factors, or environmental stimuli for an enhanced cardiomimetic model of human myocardium.

Timeline:

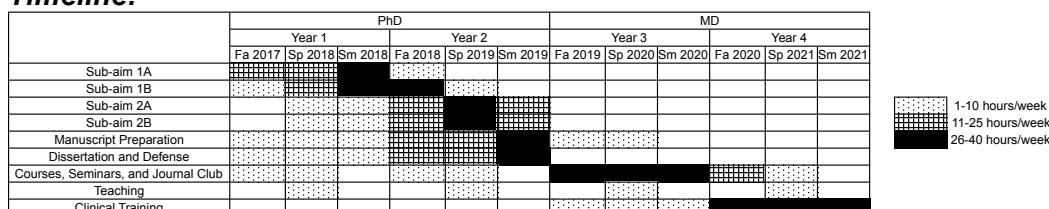


Figure 7. 4-year timeline for proposed studies and training plan. Legend is inset on right.

RESPECTIVE CONTRIBUTIONS

The Applicant and Dr. Kevin Costa developed the ideas discussed within this proposal. The Applicant wrote the entire proposal, and frequently consulted with his primary mentor, Dr. Kevin Costa, to carefully review the experimental plan, pertinent literature, and preliminary results. The Applicant also benefitted from input and feedback from co-mentor, Dr. Roger Hajjar, and advisory committee member, Dr. Susmita Sahoo. Through this process, the Applicant has strengthened his scientific knowledge, data analysis, and writing skills. The Applicant and Dr. Kevin Costa both are confident that the work proposed will build a solid foundation for the Applicant's training as a physician-scientist. To ensure this, Dr. Kevin Costa and the Applicant will meet during weekly lab meetings, as well as weekly one-on-one meetings; in addition, the Applicant will have monthly meetings with the co-Mentor, and semi-annual meetings with the Advisory Committee, for regular constructive feedback and monitoring of progress.

Preliminary data included within this proposal was generated primarily by the Applicant (multi-human engineered cardiac tissue bioreactor design in **Figs. 2**; excitation-contraction simulations in **Fig. 2**; hMSC exosome uptake by cardiomyocytes and fibroblasts in **Fig. 3**; hMSC exosome effects on hECTs contractile function and gene expression in **Fig. 4-5**; partial least square regressions model in **Fig. 5**). In addition, data was also generated by members of the Costa lab, including Dr. Irene Turnbull, M.D. (human engineered cardiac tissue construction in **Fig. 1**) and Dr. Timothy Cashman, Ph.D. (counteracting paracrine and heterocellular coupling effects of mesenchymal stem cells on hECT function in **Fig. 2**). Preliminary data was also generated with collaborators in the Cardiovascular Research Center at the Icahn School of Medicine at Mount Sinai, including Dr. Delaine Ceholski, Ph.D. (R14-del phenotype results in **Fig. 1**; gene expression results in **Fig. 4B-C** and **Fig. 5B**).

SELECTION OF SPONSOR AND INSTITUTION

As a sophomore undergraduate student, I applied to the Icahn School of Medicine at Mount Sinai (ISMMS) Summer Undergraduate Research Program, initially motivated by Sinai's reputation for strong research and clinical training. Sinai is one of the top 20 NIH-funded Medical Schools in the country, and with the seven hospitals within the expanded Mount Sinai Health System, ISMMS provides unparalleled opportunities for diverse clinical and research training. In the summer of 2012, I was selected from a competitive applicant pool to be an ISMMS Summer Undergraduate Research Fellow. Following my summer fellowship experience, my overarching motivation for selecting the ISMMS institution became much more personalized and meaningful; specifically, during this experience, I was exposed to Dr. Costa's laboratory, as well as the rigorous, creative, and flexible environment at ISMMS. This experience ultimately led me to select the ISMMS as my training institution, and Dr. Costa as my mentor and thesis advisor.

Sinai's innovative and flexible nature was immediately apparent during my summer research program. As a summer research fellow interested in applying to the ISMMS MD/PhD program, I met with the graduate school administration to express my concern of limited bioengineering graduate courses. During this meeting, I was informed by the graduate school administration that a new multi-disciplinary concentration would emerge that would fit my translational and engineering interests. Indeed, the following year, the Design, Technology, and Entrepreneurship concentration was founded at the ISMMS. This assured me that Sinai is fully dedicated to meeting student needs; furthermore, our ongoing collaboration with Dr. J. Jeremy Rice at the IBM T.J. Watson Research Center assured me that Sinai will maintain a forward-looking emphasis on integrating engineering into healthcare and biomedical research for developing novel medical solutions.

Sinai's creative and adaptable environment was further exemplified through its various early assurance programs, and its full support for students to modify the traditional timeline of MSTP training. Following my summer research fellowship, Sinai recognized my potential for applying engineering principles in the biomedical field and accepted me early assurance into its MD/PhD program. Through this early acceptance, I was not required to take the MCAT, which allowed me to focus most of my time on completing my Bachelor's and Master's degrees concurrently. Sinai's flexibility made it possible for me to be the first Cooper Union Chemical Engineering student to concurrently complete these two degrees within a four-year timeframe.

Sinai's flexibility also prompted my decision to begin my PhD training immediately after completing MD1. Following three successful rotations in Dr. Costa's lab throughout my undergraduate and graduate career, I was too excited by my research to delay my PhD training any longer; to this end, Sinai's graduate school administration fully supported my decision to start my PhD after only one year of the MD/PhD training. This is a true testament to Sinai's adaptability to meet all of its student's needs in order to provide them the best possible personalized training.

My final reason for selecting the ISMMS MD/PhD program was because of the invaluable experiences I have had with Dr. Costa throughout my undergraduate and graduate rotations. My aspiration to rotate within a lab that applies engineering approaches to biomedical research led me to contact Kevin Costa, PhD. Throughout my initial meeting with Dr. Costa, I realized that we shared similar interests, which ultimately led me to choose his laboratory for summer research. Dr. Costa's passion for his research inspired me to conduct translational cardiac tissue engineering research, an area I had not previously considered. Furthermore, Dr. Costa's mentorship, encouragement, and patience fulfilled my needs for a thesis advisor, making it an extremely easy choice for me to join Dr. Costa's lab. With Dr. Costa's office adjoining our lab space and his "open-door" policy, he is always available for discussions, questions, and advising. While providing this close advising, he also cultivates the independence necessary for graduate student training. Finally, Dr. Costa's focus on translational research fits my needs as a future physician-scientist. The lab's collaboration with Dr. Hare allows me to think about translational implications of my work, such as stem cell-based cardiac therapies. To complement this translational focus and to obtain a unique perspective on becoming a clinician-scientist, I selected Dr. Roger Hajjar as a co-mentor, who has translated his scientific work from the laboratory to clinic with his recent SERCA2a gene therapy clinical trials.

My enthusiasm to complete my thesis work in Dr. Costa's lab is clearly evident by the three successful and productive rotations I completed in his lab. Dr. Costa is an excellent mentor; he is always looking out for my best interests, and he provides me with all the tools necessary to achieve my short- and long-term goals. It is a pleasure to be a member of his lab, and I truly believe his mentorship will prepare me for a successful career as an independent physician-scientist.

RESPONSIBLE CONDUCT OF RESEARCH

Format

The Responsible Conduct of Research (RCR) course at the Icahn School of Medicine at Mount Sinai is taught by Charles Mobbs, PhD, Professor of Neurosciences and Geriatrics. All pre-doctoral students—including MSTP students—in Mount Sinai's Graduate School programs receive formal instruction in RCR during the fall of the first year. Because of scheduling conflicts, an RCR class tailored to MSTP students is offered at a different time and room than RCR for first-year PhD students. In addition to a different schedule, the course entails material more specific to MSTP students, including more emphasis on human studies in both didactic material and case studies. Attendance is mandatory. Students may not miss more than two sessions; for any session missed, the student must complete a written assignment that covers that material, and may have to review the material one-on-one with the course director.

The first year RCR course encompasses a series of presentations primarily by the course director. Lectures consist of presentations that expand on readings, bringing in concrete examples. As part of this process, the course will also include studies examining the many forms of unconscious cognitive bias that can bias conclusions. The main resource is the website created by the Office of Research Integrity (ORI), which presents official NIH positions on most of the issues described in the course. At the end of the course, students are required to spend at least 2 hours engaged in the ORI TheLab interactive video website, making various choices as the various characters and submit a report on their choices and the consequences of those choices, as well as an evaluation of the value of this experience. The students seem to find participating in this interactive video overwhelmingly positive and useful to strengthen their understanding of the material.

Additionally, outside of the course, students can attend monthly "Center-wide Ethics Luncheons," sponsored by the Department of Medical Education, which bring together physicians, scientists, students, nurses, social workers, and patient representatives to discuss ethical issues surrounding a clinical case at the medical center.

Subject Matter

The topics for these 8 sessions (2 hours each) are as follows:

1. Research Misconduct
2. Experimental Design and Data Management Practices
3. Mentor and Trainee Responsibilities; Collaborative Research
4. Conflicts of Interest; Intellectual property
5. The Protection of Human Subjects
6. The Welfare of Laboratory Animals
7. Publication, Responsible Authorship, and Peer Review
8. Grant Process and Fiduciary Responsibility

All pre-doctoral students also have specific instruction on EPA issues, fire safety, and biosafety. They are required to take advantage of the available instruction on radio-safety, animal use, or human subjects issues if these issues are part of their research work.

Faculty Participation

The first year RCR course encompasses a series of presentations primarily by the course director, Dr. Charles Mobbs. Occasional lectures are led by experts or other authorities, such as the head of the Research Integrity Officer, Dr. Reginald Miller. Reginald W. Miller, D.V.M., DACLAM, who has had a major role in administration of Mount Sinai's Animal Care facilities and been a long-standing participant in RCR education, was appointed to the position of Research Integrity Officer in the Office of the Dean a few years ago. He is overseeing a coordinated increase in RCR education at all levels of the institution that will add even greater vitality to a program that has been a long-standing institutional commitment. He also gives a presentation on an overview of the system by which Mount Sinai monitors research integrity. The material involves increasing concern about reproducibility in basic biological research, including the now-famous "shrinking effect" and how following the highest professional standards (e.g. adherence to industry standards of note-book keeping, data management, and blinded studies) should minimize this problem.

Duration of Instruction

This course has 8 sessions (2 hours each), for a total of 16 hours of instruction. The course runs during the Fall semester.

Frequency of Instruction

Lectures are once a week, for eight weeks, during the fall semester. The course will be repeated at least once every four years, and during each stage of the applicant's career.

Section II--Sponsor Information

A. Research support available

Table of current and pending research and training support specifically available to the Applicant for this particular training experience.

Source: NIH Office of the Director	ID: U54OD020353 (active)	
PI: R Cagan	Dates: 09/01/2015 - 08/31/2020	TDC: \$5,800,000
Title: <i>A New Disease Platform Leveraging Complex Drosophila and Mammalian Models</i>		
Comments: Dr. Costa is co-investigator in one project of this center grant, focused on developing personalized models of human cardiomyopathies, and he is responsible for characterizing contractile function of human engineered cardiac tissues created using iPSC-derived cardiomyocytes from patients with RASopathies. The budget for this project includes support for supplies related to human cardiac tissue engineering efforts in the Costa lab that can be used to partially support the Applicant's F30 project.		
Source: Fondation Leducq	ID: Transatlantic Network of Excellence in Cardiovascular Research (active)	
PI: D Sassoon and T Finkel	Dates: 10/01/13 - 09/30/18	TDC: \$6,000,000
Title: <i>Cellular and Molecular Targets to Promote Therapeutic Cardiac Regeneration</i>		
Comments: Dr. Hajjar is Director of a Core Project focused on taking new knowledge about the regulatory restraints on cardiac regeneration (from other projects in the network) and moving this forward in pre-clinical models by beginning to test methods to activate endogenous regeneration using small molecules or siRNA-directed therapies. The budget for Dr. Hajjar's project is \$644,776 in TDC, which includes funding for developing in vitro models of healthy and failing human heart tissue, including HECTs with Dr. Costa, for pre-clinical screening of novel cell and gene based therapies for cardiac repair. This includes funds for studying the effects of stem cells including hMSCs on cardiac function, which is directly related to the Applicant's F30 project proposal.		
Source: NIH/NHLBI	ID: R01 HL132226 (<i>pending activation on April 1, 2017</i>)	
PI: KD Costa	Dates: 04/01/17 - 03/31/22	TDC: \$1,250,000
Title: <i>Harnessing Paracrine Mechanisms of Stem Cell-mediated Cardiac Contractile Enhancement</i>		
Comments: The aims of this proposal are to evaluate the role of secreted paracrine factors in the stem cell-mediated contractility enhancement of human engineered cardiac tissues, to identify specific secreted factors and exosomes responsible for the cardiotropic effects, and to test the efficacy of isolate delivery as a therapeutic strategy for myocardial repair. Funding for stem cell culture and tissue engineering supplies will be available to support the Applicant's F30 project.		

B. Sponsor's previous fellows / trainees

Table of five representative trainees selected from a total of 8 pre-doctoral students and 6 post-doctoral fellows previously sponsored by Dr. Costa.

Name / Training Dates	Awards	Present Position
Eun Jung (Alice) Lee, PhD 2000-2006 (pre-doctoral)	2003 BMES Graduate Student Research Award	Assistant Professor of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ
Do Eun Kim, PhD 2004-2009 (pre-doctoral)	2004-2008 Samsung Foundation Scholarship; 2008 SPRBM Grad Student Presentation Award	Engagement Manager, IMS Consulting, New York, NY
Evren U. Azeloglu, PhD 2004-2009 (pre-doctoral)	2004, 2005 Stony Wold-Herbert Fund Graduate Fellowship; 2009 Yuen-huo Hung & Chao-chin Huang Award in Biomedical Engrg.	Assistant Professor of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY
Jia-Jye Lee, PhD 2010-2015 (pre-doctoral)	2014, 2015 Stony Wold-Herbert Fund Graduate Fellowship	Postdoctoral Fellow in Biomedical Engrg., University of Virginia, Charlottesville, VA
Timothy Cashman, PhD 2011-2015 (pre-doctoral)	2013-2016 NIH/NHLBI F30 Individual Predoctoral MD/PhD Degree Fellowship	Fourth-year Medical Student in MD/PhD Program, Icahn School of Medicine at Mount Sinai, New York, NY

Table of five representative fellows and trainees selected from a total of 8 pre-doctoral students and more than 40 post-doctoral fellows previously sponsored by Dr. Hajjar.

Name / Training Dates	Awards	Present Position
Federica del Monte, MD, PhD 1998-2003 (post-doctoral)	2002 NHLBI K08 Mentored Clinical Scientist Development Award	Associate Professor of Medicine, Harvard Medical School, Principal Investigator, Beth Israel Hospital, Boston, MA
Djamel Lebeche, PhD 1999-2006 (post-doctoral)	2004 NHLBI K01 Mentored Research Scientist Development Award	Associate Professor of Medicine (Cardiology), Icahn School of Medicine at Mount Sinai, New York, NY
Ioannis Karakikes, PhD 2007-2012 (post-doctoral)	2009 Louis B. Mayer Foundation Award; 2012 NHLBI K99/R00 Pathway to Independence Award	Assistant Professor, Department of Medicine (Cardiovascular Medicine), Stanford University School of Medicine, Palo Alto, CA

Changwon Kho, PhD 2007-2013 (post-doctoral)	2013 NHLBI K99/R00 Pathway to Independence Award	Assistant Professor of Medicine (Cardiology), Icahn School of Medicine at Mount Sinai, New York, NY
Thomas LaRocca, MD, PhD 2008-2011 (pre-doctoral)	2009 NHLBI F30 pre-doctoral fellowship	Medical Resident, Department of Pediatrics, UC San Francisco, CA

C. Training plan, environment, and research facilities

Training Plan for Joshua Mayourian.

The training Plan of this F30 proposal has been crafted to: 1) build on Josh's existing strengths in engineering and previous research experiences; 2) nurture his intellectual creativity; 3) foster his interests in stem cell-based cardiac regenerative medicine; and 4) provide in-depth training in stem cell biology, cardiac (patho) physiology, cell signaling and tissue engineering. This Training Plan will prepare Josh with the tools to pursue an investigative career at the cutting edge of developing therapies for cardiac repair and regeneration.

The Training Plan is structured around recognized success metrics for a career as a translational biomedical researcher, and includes the following elements: 1) acquisition of "laboratory bench" competencies, 2) structured didactic program, 3) interactions with the primary sponsor, Dr. Costa, co-sponsor, Dr. Hajjar, and Advisory Committee Members, and 4) training in professional survival skills.

1) Acquisition of Technical Competencies Technical competencies will include the following, with associated advisory team experts:

- a. Healthy and diseased human cardiac tissue engineering (Costa)
- b. Cardiac stem cell differentiation and pluripotent stem cell biology (Dubois, Hajjar, Costa)
- c. Mesenchymal stem cell biology and cardiomyocyte interactions (Kovacic, Hajjar)
- d. Cardiac excitation-contraction coupling (Akar)
- e. Cardiac microstructure and gene expression profiling (Costa, Hajjar)
- f. Cardiovascular paracrine signaling and exosomes (Sahoo)
- g. Cardiac mRNA and therapeutics (Hajjar)

2) Structured Didactic Program, Josh will attend courses and seminars offered through the Graduate School of Biomedical Sciences at Mount Sinai (ISMMS). He will also take advantage of a graduate training partnership with the Biomedical Engineering Department at the neighboring City College of New York (CCNY). He has already completed the Quantitative Graduate Physiology course (ISMMS) identified in the original proposal. Two additional courses that will be taken in year 1 of the training plan are briefly described below.

a. To build on the first year of medical school and the required core courses from the PhD, Josh will enroll in two additional **formal courses** as technical electives in Cell Signaling Systems (ISMMS) and Cell and Tissue Engineering (CCNY).

- **BSR 2802: Cell Signaling Systems (ISMMS)** This course will provide an introduction to the molecular details and systems perspective of cellular signaling systems, including G-protein coupled receptors, apoptotic pathways and regulation of transcription machinery by signaling pathways and ion channels. Systems to be analyzed include a developmental model. This information will be relevant to understanding the signaling pathways related to cardiac contractility, hypoxia, and myocyte-nonmyocyte interactions, as directly relevant to the proposed research.

- **BME I2000: Cell and Tissue Engineering (CCNY)** This course focuses on the application and design of cellular and biomaterial microstructures for biomedical applications. Topics include: matrix molecules and their ligands, construction of biomimetic environments, biomaterials for tissue engineering, tissue engineering in bone and cartilage, and genetic approaches in cell and tissue engineering. The material learned in this course will provide a solid foundation in the principles governing tissue engineering research, and will help develop a deeper understanding and broader awareness of the field.

b. Josh will attend **weekly seminars** presented by international experts via the Cardiovascular Research Center (CVRC) Seminar Series and selected seminars from the Black Family Stem Cell Institute Seminars in Developmental and Regenerative Biology, the Biophysics and Systems Pharmacology Seminars, and a variety of other regular weekly seminars at ISMMS. Josh will also attend weekly Cardiovascular Institute Grand Rounds, Advanced Heart Disease Seminars, and monthly Controversies in Cardiology to reinforce clinical perspectives while his research efforts are focused in the lab.

- c. To learn about **commercial aspects of translating biomedical discovery**, Josh will attend a quarterly Intellectual Property and Commercialization Speaker Series, sponsored by Mount Sinai Innovation Partners (MSIP), the technology transfer office at Mount Sinai. He will also attend selected monthly NYC TechConnect "Riverside Chats" seminar and networking series focused on developing bioscience entrepreneurship, held at various locations throughout Manhattan.
- d. Continued training in the **Responsible Conduct of Research and Bioethics** will occur by attending annual and semi-annual refresher courses at ISMMS. Related topics are also regularly addressed as they arise during interactive discussions in weekly Costa Lab meetings and journal club.

3) Interactions with Sponsors and Advisory Committee In addition to impromptu interactions in Dr. Costa's laboratory or office (which opens directly into the lab) regular scheduled meetings will be as follows:

- a. Josh will continue meeting with Dr. Costa in weekly one hour sessions for reviewing latest results, planning new experiments, considering alternative approaches, preparing manuscripts and conference abstracts, and general research mentoring. The co-sponsor, Dr. Hajjar, will join these meetings on a monthly basis. Four times per year, Josh will prepare a written report summarizing his research accomplishments, revisiting target milestones, and outlining forthcoming manuscripts. Reviewing drafts of manuscripts to be submitted will be handled with co-authors in separately designated sessions.
- b. Josh will meet semi-annually with a group of advisors who will focus not only on results of the research project but also on aspects of development as a clinician-scientist. In addition to the primary sponsor, the group will include co-sponsor Roger Hajjar, MD (cardiac gene therapy and heart failure; establishing a career as clinician-scientist), Jason Kovacic, MD, PhD (cardiovascular stem cell biology; clinician/researcher balance), Fadi Akar, PhD (cardiac electrophysiology), Susmita Sahoo, PhD (exosomes and cell-cell signaling), and Nicole Dubois, PhD (stem cells and cardiac development). The meeting format will include a formal 20-30 minute progress update on the proposed research and associated career development activities (e.g., manuscript preparation). A question and answer session will follow. Drs. Kovacic, Akar, Sahoo, and Dubois will prepare a brief written report on overall progress, areas for improvement and other recommendations, to be presented to Drs. Costa and Hajjar for review with the Applicant.

4) Professional Survival Skills (Oral Presentations and Professional Writing)

- a. Josh will continue to make regular oral presentations in the CVRC Research Seminars (1/yr), Costa Lab Journal Club (4/yr), Biophysics and Systems Pharmacology (BSP) Works in Progress (1/yr), as well as poster presentation at the annual MSTP Research Day. Attendance and presentation of research at a scientific meeting such as AHA Basic Cardiovascular Science (BCVS), Tissue Engineering and Regenerative Medicine International Society (TERMIS), Biophysical Society (BPS), or Biomedical Engineering Society (BMES) is also expected at least once per year.
- b. Josh will prepare a complete first draft of conference abstracts and manuscripts resulting from his work.
- c. Development of a career as a clinician-scientist will be addressed with Drs. Hajjar and Kovacic, who offer the perspectives of established and early career investigators, respectively, as described above.
- d. Preparing applications for research support will be learned via interactions with Drs. Costa and Hajjar, and by attending Grant Writing Workshops and Lectures offered through the ISMMS Graduate School and the Cardiovascular Institute (CVI) Postdoctoral Mentoring Program (PMP). A formalized mentoring program conceived by Dr. Martin Schwarz, Dr. Hajjar and CVI Director, Dr. Valentim Fuster and initiated in 2009, the CVI-PMP provides participants with information regarding funding opportunities, the mechanics of constructing an application for extramural support, and the peer-review process. Topics on career development are routinely discussed, and Josh will attend the program. The preparation of this F30 proposal has itself been a superb training opportunity; Josh completely wrote the application, with minor editing and guidance from Dr. Costa and Dr. Hajjar during initial planning and revision processes.

Research and Training Environment at Mount Sinai

Mount Sinai Medical Center includes Mount Sinai Hospital, the School of Medicine and the Graduate School of Biological Sciences. Spanning the blocks between 98th Street and 102nd Street on the border of East Harlem in Manhattan, Mount Sinai consistently ranks among the top 20 medical schools in the United States both in NIH funding and by US News and World Report. Noted for innovation in education, biomedical research, clinical care delivery, and local and global community service, the research environment at Mount Sinai is translational, collaborative, and state-of-the-art, with a wealth of shared facilities, and an impressive breadth of

expertise among more than 5,000 faculty in 33 departments and 32 research institutes. This includes Mount Sinai Heart and the Cardiovascular Institute (CVI), directed by Dr. Valentin Fuster, one of the world's most respected and widely recognized leaders in cardiovascular medicine. Within the Cardiovascular Institute is the CVRC, directed by co-sponsor, Dr. Roger Hajjar, a world-renowned expert in heart failure and gene therapy. The CVRC consists of 9,000 square feet of prime wet lab research space with 8 PIs and over 40 postdoctoral fellows, with expertise spanning stem cells, gene therapy, heart failure, metabolism, electrophysiology, biomechanics, tissue engineering, regenerative medicine, nanomedicine, and large animal surgical facilities for preclinical studies. The Costa Laboratory for Cardiovascular Cell and Tissue Engineering is housed within the CVRC. Research in the Costa lab focuses on understanding the mechanobiology of cardiovascular cell and tissue function in healthy and diseased states. Computational modeling is combined with state-of-the-art experimental methods to attack this challenging multi-scale problem. Atomic force microscopy (AFM) is used to map the regional micro-scale viscoelastic properties of isolated cells and intact tissues, and to examine the response to targeted mechanical stimulation, using finite element analysis to guide the experiments and enhance the information that can be extracted from AFM indentation tests. Functional 3D engineered cardiac tissues and organoid chambers are created for investigating mechanisms of cardiovascular remodeling, injury, repair, and regeneration. These living model systems, created from human pluripotent stem cell derived cardiomyocytes, allow unprecedented control of tissue composition, structure, and geometry combined with advantages of long-term viability, species specificity, and high-throughput screening capability. This multidisciplinary research and training environment offers a wealth of opportunity for interaction with other scientists and groups both within and beyond the CVRC, providing a fertile environment for Josh's MD/PhD training. Indeed, development of the next generation of physician scientists and researchers in cardiovascular medicine is a priority of the CVRC and the CVI, as evidenced by the above CVI-PMP mentoring program.

The Medical Scientist Training Program (MSTP) at Mount Sinai is strongly committed to providing guidance and mentorship to students throughout all phases of their MD/PhD training. The Institutional Commitment to Training states that students obtain guidance and advice from the Program Director (Dr. Margaret Baron), as well as from Associate and Assistant Directors, including yearly Progress Meetings to help keep MSTP students on track. In addition, several formal venues provide support pertinent to a physician-scientist's training and career. These include: 1) Annual MD/PhD Off Campus Retreat, where Alumni, Directors of ISMMS's post-graduate training areas and Graduate Faculty have interactive discussions with MSTP students regarding various issues related to graduate training, residency and career options; 2) monthly Medical Scientist Research Seminar (MSRS) dinners with presentations by Graduate School faculty and MSTP graduates who not only present their research, but also provide an overview of their career path. A major highlight is the MSRS session devoted to "Life after MSTP Training: Residency and Beyond" where graduating MSTP students, as well as recent graduates in their first house-staff, fellowship, and/or junior faculty position, discuss residency and fellowship selection and application processes; and 3) Mount Sinai students host the annual North East Regional Meeting of the American Physician Scientist Association (APSA) to promote interactions with fellow MD/PhD students and eminent senior physician-scientist role models outside Mount Sinai.

The MSTP program at ISMMS has been specifically designed to enhance integrative, translational training throughout all phases of student education towards the combined degree. Aside from the normal PhD training, MSTP students have multiple opportunities to integrate their medical and doctoral training through elective courses, *Clinical Case Discussions*, a monthly journal club providing MSTP students continued exposure to the practice of clinical medicine, and the required *Clinical Refresher Course* in the last year of the PhD phase to improve the clinical performance of our MSTP students when they return to medical school. Altogether, these and other educational and career guidance opportunities provide a firm foundation for students to be extremely well prepared to become physician-scientists who are strong both clinically and scientifically.

Available Research Facilities

As detailed in the Resources section, essential facilities and equipment required for the proposed studies are available and functional in the Costa laboratory, and in the CVRC directed by co-sponsor, Dr. Hajjar. This includes facilities for bioreactor design, cardiac tissue engineering, muscle function testing, qRT-PCR, exosome extraction and analysis, cryosectioning, and confocal microscopy. ISMMS Core facilities for human stem cell culture, high throughput screening, histology, and optical and electron microscopy are also available.

D. Number of fellows / trainees to be supervised during the fellowship

In addition to the applicant, Dr. Costa anticipates supervising up to two doctoral students and two postdoctoral fellows, plus up to three post-graduate researchers or masters students that typically constitute the Costa lab.

This lab size has proven effective to allow personal interaction between Dr. Costa and each lab member, to establish a hierarchy of supervision within the lab, and to provide a diversity of backgrounds and expertise to tackle multi-disciplinary problems that are hallmarks of the biomedical engineering approach to research. This is complemented by Dr. Hajjar's lab, which typically has approximately 20 postdoctoral fellows and trainees.

E. Applicant's qualifications and potential for a research career

Joshua Mayourian is an exceptional candidate for an F30 Individual Predoctoral NRSA for MD/PhD Fellowship, and has outstanding potential for an investigative career as a physician-scientist. Josh is currently in his third year in the MSTP program at Mount Sinai and, as described below, he decided to depart from the traditional MD/PhD schedule by postponing medical school and beginning doctoral studies full-time after his first year, motivated by an ideal timing situation for his thesis project. Josh is majoring in the Biophysics and Systems Pharmacology (BSP) graduate training area, where he maintains an impressive 3.9 GPA. Before joining Mount Sinai, Josh graduated *summa cum laude* from The Cooper Union, where he majored in Chemical Engineering, minored in Biomedical Engineering, and also earned a Master of Engineering degree, all within a 4-year timeframe. Thus, Josh's superb academic performance has consistently reflected his strong intellect.

I, Kevin Costa, have known Josh as his research mentor since June, 2012, when he began working in my lab through the Summer Undergraduate Research Program (SURP) at Mount Sinai. Only a sophomore, Josh impressed me with his interest and ambition to identify a project related to cardiac tissue engineering that he could call his own. Josh took on the project of building a novel bioreactor device that allows side-by-side culture of multiple ECTs and functional testing of twitch force and beating frequency, with added features of reduced tissue size for more efficient use of valuable cardiac cells. Given an initial concept, Josh designed the bioreactor using Solidworks simulation software, and worked with a machinist at Cooper Union to fabricate the device, which he then tested. It was an impressive initial prototype that was submitted as an invention disclosure to MSIP, though it was ultimately not patentable. My lab subsequently refined the design to create an apparatus that enabled several high priority projects, including Josh's current PhD thesis research.

Josh also demonstrated strong analytical abilities when he took the extra step of developing a mathematical model to predict how the concentration of secreted soluble factors would be expected to change with time as the tissues were cultured in his bioreactor. With his formal education in molecular transport processes mostly still ahead of him in school, Josh impressed me with his ability to reason out the expected shapes of the concentration curves before working through some fairly challenging mathematics to obtain the governing equations, which he implemented in MatLab. Josh also performed the experimental measurements to validate his model and predict the biologically relevant rates of cytokine secretion and uptake. Josh made great progress with this project, and his first-author abstract was accepted for oral presentation in an Undergraduate / REU session of the 2012 *Annual Biomedical Engineering Society* meeting, for which Josh traveled to Atlanta for his first presentation at a national scientific meeting.

Based on his success, I welcomed Josh back to my lab in summer 2013 for a second SURP rotation. This time he focused on understanding the electrophysiological consequences of MSC-CM coupling, which is a critical research area related to stem cell therapies for the heart. Building on a published model of the interaction between human cardiac fibroblasts and myocytes, Josh adapted the fibroblast model to represent the electrophysiological properties of mesenchymal stem cells. He wrote his own MatLab and Python code to implement the model on the Sinai Supercomputer, and based on published experimental patch clamp data, he created three different phenotypic hMSC families based on their expressed ion channels, fit the relevant equations to data extracted from the published figures, and simulated the effects of varying the hMSC:hCM ratio to mimic the conditions that might be found locally during cell implantation therapies in the heart. The findings demonstrate that one particular class of hMSCs is notably less disruptive of the coupled action potential, and thus would be predicted to have the least potential for negative arrhythmogenic consequences. Thus, the model recommends a modification of current therapeutic strategies in clinical trials by pre-sorting for this hMSC sub-type.

During that summer, Josh also managed to resurrect a defunct patch clamp system that had been sitting unused, sought advice from faculty and postdocs with patch clamping expertise at Mount Sinai and neighboring institutions, and successfully obtained measurements of hMSC electrophysiology that were consistent with the published data. Though this was of limited scientific novelty, the fact that Josh had the determination and skill to succeed with such demanding single-cell experiments, and appreciated the value of

performing the measurements to better understand what he was mathematically modeling, showed an impressive level of scientific insight and maturity.

Josh was subsequently admitted early decision to the MSTP program at Mount Sinai through FlexMed, freeing his senior year to satisfy the requirements for a combined ME/BE degree from Cooper Union and completing his mathematical modeling studies as his Master's thesis. His resulting manuscript was published earlier this year as a first-author paper and cover article in *PLoS Computational Biology*.

After completing first year of medical school and other research rotations, Josh decided to pursue his PhD training with me, and returned to the lab in summer 2015. Josh has taken over a project from another MSTP student and NIH F30 trainee, Timothy Cashman, who defended his doctoral dissertation in May, 2015, and has since returned to medical school. Josh quickly became independent with the stem cell differentiation techniques and cardiac tissue engineering methodology necessary to make immediate progress with his dissertation research. Josh began collaborating with Dr. Susmita Sahoo at Mount Sinai to study the role of exosomes in MSC-mediated paracrine signaling in the heart, which is an identified topic of high priority for NHLBI. This collaboration generated critical preliminary data for my resubmitted NHLBI R01 to support this research, which received a 12 percentile score and is anticipated to commence funding in April, 2017. Fostering Josh's computational interests, we also initiated a collaboration with Dr. Jeremy Rice, manager of the Multiscale Systems Biology and Modeling Group at IBM Watson Research Center just outside Manhattan, and Josh spent 10 weeks during Summer 2016 as an intern working on a 3D anatomically detailed finite element model of the human heart to better understand cardiac contractility and arrhythmogenicity. Josh concurrently extended his original heterocellular coupling model to include paracrine signaling effects of hMSCs, and demonstrated a surprising compensatory effect of the paracrine factors that counteracts potential risks of direct hMSC-cardiomyocyte coupling. Josh was able to simulate the conditions of our earlier studies of hMSC-supplemented hECTs, and his model did a remarkable job of matching key experimental findings, validating the approach. This combined modeling and experimental study has been submitted for peer review with Josh as first author.

Josh was largely independent in drafting this F30 resubmission application, which is directly related to his doctoral thesis that he successfully proposed to his thesis committee on December 10, 2015. During the past year, Josh has worked tirelessly to generate the compelling preliminary data in this resubmission proposal, effectively completing most of the original F30 proposal aims, and generating an interesting hypothesis about the role of MIR-21 as an essential component of hMSC-exosomal cargo for enhancing cardiac contractility. Also, in response to the original reviewer critiques, he redirected his disease modeling efforts toward the more relevant condition of non-ischemic cardiomyopathy, which is a topic of high significance for human health.

Josh is currently funded by a competitive NIH T32 institutional training grant in Biophysics and Systems Pharmacology (BSP). Because timing of this project was perfect to make rapid and impactful progress, Josh elected to begin full-time doctoral research a year earlier than the typical MSTP student. This also has advantages for his medical school training, allowing Josh to proceed directly from year 2 of medical school into clinical rotations in year 3. In addition to consulting with myself and co-sponsor, Dr. Hajjar, Josh made this strategically important decision with input from Dr. Kovacic (Advisory Committee member), Dr. Yasmin Hurd (former MSTP director), and Dr. Cashman (senior MSTP lab mate), demonstrating a cohesive mentoring team and a flexible, individualized training program.

In summary, I am delighted to support this F30 MD/PhD Fellowship application for Josh Mayourian. Josh's interest and intuition for engineering combined with his focus on problems of biological and medical relevance suggest he could become the kind of technologically sophisticated physician scientist that is certain to be at the forefront of medical discovery in the future. I have been fortunate to have some outstanding students in my lab over fifteen years in academia (including Goldwater Scholarship winners and Rhodes Scholarship finalists), both here at Mount Sinai and previously at Columbia University, and I would rank Josh in the top 1% as one of the very best. I am certain he has what it takes to develop into a leading physician-scientist, combining his passion and expertise in mathematics, biological sciences, engineering, and medicine, who will work to discover new breakthroughs at the forefront of the burgeoning fields of stem cell biology and tissue engineering for applications in cardiology research and therapy..



**Mount
Sinai
Heart**

Roger J. Hajjar, MD
*Director, Cardiovascular Research
Arthur & Janet C. Ross Professor of Medicine, Cardiology*

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roger.hajjar@mssm.edu

December 6, 2016

Dear Joshua,

Thank you for asking me to serve as a co-sponsor for your fellowship application entitled "The Role of Exosomes in Mesenchymal Stem Cell-Mediated Enhancement of Cardiac Contractility." As Director of the Cardiovascular Research Center (CVRC) at the Icahn School of Medicine at Mount Sinai and with over two decades of active clinical and research experience, I can provide a unique perspective on how to become a successful clinician-scientist. I also have experience in translating my scientific research from bench to bedside as demonstrated by the recent SERCA2a gene therapy clinical trials.

Over the past year, it has been a pleasure meeting with you to discuss your exciting preliminary data on the effects of exosomes on your human engineered cardiac tissues. I am confident your continued participation in the Cardiovascular Research Center's journal and data clubs will further enhance your training as a graduate student.

With Dr. Kevin Costa as primary sponsor on the proposal, I am confident you will be conducting exciting research in human mesenchymal stem cell biology and cardiac tissue engineering. You have surrounded yourself with both the scientific and research support needed to successfully complete the proposed project.

Sincerely,

Roger J. Hajjar, M.D.



Susmita Sahoo, PhD

Assistant Professor | Cardiovascular Research Center
Icahn School of Medicine at Mount Sinai
Phone: 212.824.8913 | Fax: 212.241.4080 | Email: susmita.sahoo@mssm.edu
One Gustave L. Levy Place, Box 1030
New York, NY 10029

5 December 2016

Dear Joshua,

I am delighted to serve as a Collaborator and an Advisory Committee Member for your F30 proposal entitled, "The role of exosomes in mesenchymal stem cell mediated enhancement of cardiac contractility". I am impressed by your scientific approach using human engineered cardiac tissues to tease out the paracrine signaling effects of mesenchymal stem cells on cardiomyocyte contractility, and by the compelling preliminary data we have generated in collaboration with the Costa Laboratory suggesting exosomes are likely to play a key role in this process. Your scientific vision to combine the emerging field of stem cell exosomes research with high clinical significance and innovative tissue engineering approaches will open up new research avenues for scientific interrogation.

As you are aware, my laboratory in the Cardiovascular Research Center at Mount Sinai specializes in the investigation of exosomes signaling in cardiovascular disease, and we are fully equipped to help support the exosomes-related aims described in your proposal. In addition, my formal training in bioengineering provides an ideal background for collaborating on your proposed experiments, which integrate technically sophisticated methodologies including human stem cells, tissue engineering, microarray technology, and bioinformatics analysis.

I also look forward to participating in your Advisory Committee meetings, where I can provide feedback and guidance not only on your scientific progress, but also on your career development. Whether during semi-annual meetings, or informally any time you are seeking mentorship, I would be more than happy to share my advice and point of view.

I wish you complete success with this excellent F30 proposal. I very much look forward to working with you on this project and to supporting your career development. I hope that this grant support from NIH is the first step in your physician-scientist career evolution.

Sincerely,

A handwritten signature in blue ink that reads "Susmita Sahoo". The signature is fluid and cursive, with "Susmita" on top and "Sahoo" on the bottom, both underlined.

Susmita Sahoo, PhD



Joshua M. Hare, M.D., F.A.C.C., F.A.H.A.

Chief Sciences Officer

Senior Associate Dean for Experimental and Regenerative Therapeutics

Louis Lemberg Professor of Medicine

Director, Interdisciplinary Stem Cell Institute

December 7, 2016

Dear Joshua,

It is my pleasure to be a collaborator for your F30 proposal entitled, "The Role of Exosomes in Mesenchymal Stem Cell-Mediated Enhancement of Cardiac Contractility." I am very excited by the data you have gathered from the human mesenchymal stem cells (hMSCs) we have sent you, leading to co-authorship on your first author manuscript in preparation.

I am delighted to continue to send you the cells as needed to study the effects of exosomes on human engineered cardiac tissue contractility.

Good luck with the F30 application, and I look forward to continuing our collaboration.

Sincerely,

A handwritten signature in black ink that reads "Joshua Hare".

Joshua Hare

Interdisciplinary Stem Cell Institute • Leonard M. Miller School of Medicine
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INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING

The Medical Scientist Training Program (MSTP) at Mount Sinai is strongly committed to providing guidance and mentorship to students throughout all phases of their MD/PhD training. The Institutional Commitment to Training states students obtain guidance and advice from the Program Director, as well as from Associate and Assistant Directors, including yearly Progress Meetings to help keep MSTP students on track. On the following page is a description of the MD/PhD program at the Icahn School of Medicine at Mount Sinai (ISMMS) from our program director, Margaret H. Baron, MD PhD, indicating both my progress and status according to the program's timeline.

The ISMMS Cardiovascular Research Center (CVRC) is a well-established research program related to the applicant's area of interest. The multidisciplinary research and training environment in the CVRC offers a wealth of opportunity for interaction with other scientists and groups, providing a fertile environment for the applicant's MD/PhD training. The CVRC is directed by my co-Sponsor, Dr. Roger Hajjar, a world-renowned pioneer in heart failure and gene therapy. The CVRC consists of 8 PIs and over 40 postdoctoral fellows, with expertise spanning stem cells, exosomes, modRNA, gene therapy, heart failure, metabolism, electrophysiology/optical mapping, biomechanics, tissue engineering, regenerative medicine, nanomedicine, and large animal surgical facilities for preclinical studies. The Costa Laboratory for Cardiovascular Cell and Tissue Engineering directed by my Sponsor, Dr. Kevin Costa, is part of the CVRC. The development of the next generation of physician scientists and researchers in cardiovascular medicine is a priority of the CVRC and the Cardiovascular Institute (CVI), as demonstrated by the Postdoctoral Mentoring Program (see Sponsor and Co-Sponsor Information for more details).

CVRC facilities and resources will be provided for career development and the research proposed in this application. As detailed in the Resources section, essential facilities and equipment required for the proposed studies are available and functional in the Costa laboratory, and elsewhere in the CVRC. This includes facilities for bioreactor design, cardiac tissue engineering, muscle function testing, optical mapping of calcium transients, qPCR, exosome extraction and analysis, cryosectioning, and confocal microscopy. Furthermore, the ISMMS has core facilities available for human stem cell culture, high throughput screening, histology, and optical and electron microscopy. The applicant will meet semi-annually with a group of advisors who will focus on aspects of development as a clinician-scientist, as well as the results of the research project. In addition to the primary Sponsor, the group will include co-Sponsor Roger Hajjar, MD (cardiac gene therapy and heart failure; establishing a career as clinician-scientist), Jason Kovacic, MD, PhD (cardiovascular stem cell biology; clinician/researcher balance), Fadi Akar, PhD (cardiac electrophysiology/optical mapping), Susmita Sahoo, PhD (exosomes/cell-cell signaling), and Nicole Dubois, PhD (stem cells and cardiac development). Development of a career as a clinician-scientist will be addressed with Drs. Hajjar and Kovacic, who offer the perspectives of established and early career clinician-investigators, respectively.

The applicant will be exposed to numerous opportunities for intellectual interactions with other investigators during courses, journal clubs, seminars, and presentations. To build on the first year of medical school and the required core courses from the first two years of PhD, the applicant will enroll in at least two additional formal courses as technical electives Cell Signaling Systems (ISMMS) and Cell and Tissue Engineering (City College of New York). For more details on these course selections, see Sponsor and Co-Sponsor Information. The applicant will also attend weekly seminars presented by international experts via the CVRC Seminar Series, and selected seminars from the Black Family Stem Cell Institute Seminars in Developmental and Regenerative Biology, the Systems Pharmacology Seminars, and a variety of other regular weekly seminars at ISMMS. The applicant will also attend weekly Cardiovascular Institute Grand Rounds, Advanced Heart Disease Seminars, and monthly Controversies in Cardiology to reinforce clinical perspectives while his research efforts are focused in the lab. The applicant will also make regular oral presentations in the CVRC Research Seminars (once per year), Costa Lab Journal Club (four times per year), Design, Systems Pharmacology Works in Progress (once per year), as well as poster presentation at the annual MSTP Research Day. Attendance and presentation of research at a scientific meeting such as AHA Basic Cardiovascular Science (BCVS), Tissue Engineering and Regenerative Medicine International Society (TERMIS), Biophysical Society (BPS), or Biomedical Engineering Society (BMES) is also expected at least once per year.

Description of MSTP Program at Mount Sinai

December 4, 2016

Joshua Mayourian matriculated into the MD/PhD Program at the Icahn School of Medicine at Mount Sinai (ISMMS) in 2014 and is expected to graduate in 2022. The ISMMS Medical Scientist Training Program (MSTP) is committed to training future physician-scientists in an integrated program of medical and graduate education in an environment that promotes cutting-edge research for a comprehensive basic science PhD degree. A cross-disciplinary approach is taken to address complex biomedical problems, promote collaborations throughout the institution, and expose students to innovative translational initiatives. The MD/PhD program was established in 1971 and funded as an MSTP in 1977. There are 97 students in the program, with ~12 students accepted each year from an application pool of over 400 candidates. Approximately 90% of students complete the full program (past 15 years). The few students who do leave the program tend to matriculate into Mount Sinai's MD-only program. Of the graduates who have completed the program, more than 80 percent are currently pursuing careers in biomedical research.

ISMMS MSTP students earn both degrees in ~7.8 years. Students begin the program during the summer prior to year 1 (MD1) of classes in order to complete their first full time laboratory rotation. They typically spend the first two years completing the medical school basic science and the graduate school core curricula. Second and third laboratory rotations are completed during the summer between the first and second year of medical school. After MD2 and completion of Step 1 of the Boards, students enter the laboratory full time and complete the remaining graduate school courses until they earn their PhD. Students take 72 total course credits towards their PhD. Upon completing the PhD, students return to medical school for their third and fourth year of clinical training.

MD/PhD students are required to meet several milestones. By the end of the second summer, students are expected to choose a dissertation advisor and declare a training area of study. All students are required to take a course in Responsible Conduct of Research; Josh took this course in Fall, 2015. A Thesis Proposal is submitted by the end of the sixth semester. Josh defended his thesis proposal, "The role of exosomal miRNA-21 in mesenchymal stem cell mediated enhancement of cardiac contractility" in December, 2015. He is currently in the second year of the PhD phase of the program, in the laboratory of Kevin Costa, PhD. Most students successfully defend and deposit the PhD dissertation during the twelfth semester after matriculation (year 6 of the program). To facilitate the transition back to the clinic, students are required to successfully complete a graded "Clinical Refresher" course. The course incorporates didactic sessions and clinical sessions within outpatient and inpatient settings, with individualized preceptors (see Swartz and Lin, Med. Teach. 36:475-479, 2014). During clinical training, significant elective time is integrated into the curriculum to allow students to participate in research that will help them to finalize research projects and scientific papers. Josh expects to return to clinical training in 2020.

Students are monitored closely throughout their training by MSTP leadership. Students are assigned a preclinical advisor when they first start the program, to provide guidance on appropriate laboratories for thesis projects and on navigating the training program. Each student is required to meet with the Director of the program annually to discuss individual progress. They complete an Individualized Development Plan that maps out the work they have completed and the goals they anticipate going forward. Students have open access to the Director and two Associate Directors for support and guidance throughout the program and interact on a regular basis with the MSTP Leadership, through multiple opportunities for one-on-one and group discussions. These include the monthly dinnertime Medical Scientist Grand Rounds and Annual MSTP Retreat.



Icahn
School of
Medicine at
**Mount
Sinai**

Medical Scientist
Training Program
(MD/PhD)

Margaret H. Baron, MD PhD
Director, MD/PhD (MSTP) Program
Senior Associate Dean for Education

RESOURCE SHARING AND DATA DISSEMINATION PLAN

There are no new unique lines of cells or mutant animals being developed for this proposal. If during the course of the study new resources are developed, we will share these resources in a prompt manner. If during the course of the study new mathematical models or computational tools are developed, we will also share these resources in a prompt manner. Novel developments in the exosome field will be appropriately shared with the ExoCarta (<http://exocarta.org/>) exosome protein, RNA, and lipid database.

Additionally, Mount Sinai and our laboratory will comply with NIH Grants Policy on Sharing of Unique Research Resources, including the "Sharing of Biomedical Research Resources: Principles and Guidelines for Recipients of NIGH Grants and Contracts" issued in 1999 (http://www.ott.nih.gov/policy/rt_guide_final.html). Should any intellectual property be created during the course of the study that requires a patent, we will ensure that the technology remains widely available to the research community in accordance with the NIH Principles and Guidelines document through Mount Sinai Innovation Partners, the technology and business development office at Sinai. Research findings will be presented at national or international conferences and published in a timely manner. Once published, experimental results including original and analyzed data will be made publically available upon request. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central according to NIH guidelines.