Emory+Children's Pediatric Research Center

An Atlanta-based research alliance

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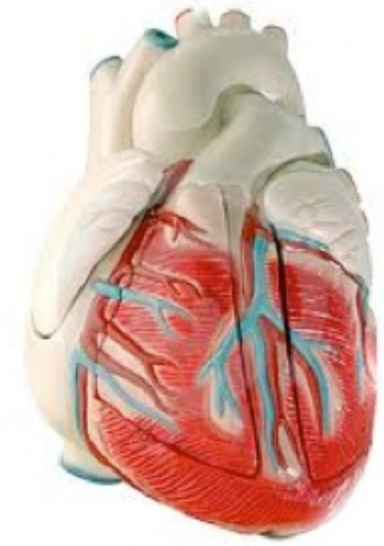


Center for Cardiovascular Biology Newsletter

Second Edition
July 2014

Director Welcome, Dr. Mike Davis

The last 6 months have brought about exciting changes in the center. We have welcomed one new faculty member (Dr. Changwon Park), another faculty member has agreed to join Emory (Dr. Hee Cheol Cho), and we have welcomed several existing Emory faculty in to our Center (Dr. Pamela Winterburg, Dr. Christopher Petit). Several new grants have been awarded in the areas of nanotechnology, progenitor cell therapy, and pacemaker engineering. In addition, we continue our strong programs in stem cell biology, neuroprotection, electrophysiology, and patient outcomes. We are building strong collaborations across campuses and within our own and feel like our investments from the past few years is really paying dividends. We'd like to thank our researchers, our doctors, and administrative staff for the success of this year, as well as those donors who helped foster our research goals. With our new and anticipated growth over the next two years, we hope you will share in our success.

Donor Appreciation

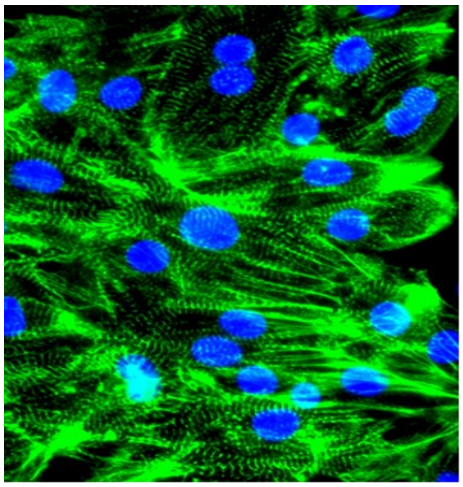
The CCB would like to recognize some of our donors who have generously provided resources to perform cutting edge research. Thanks to the philanthropy of Katrina Ceccoli and the Darryll M. Ceccoli Pediatric Cardiac Research Fund, the laboratories of Drs. Mary Wagner and Michael Davis are investigating whether progenitor cell therapy can help children with heart failure. Funds directed are being used to isolate cells from Sibley patients and treat juvenile animals with heart failure to generate data for an eventual application for a Phase I clinical trial. The CCB would also like to thank the Urowsky and Sahr families who directed a generous gift to help us recruit a well-funded and cutting edge scholar from Cedars-Sinai who has created technology to help children with arrhythmias potentially avoid pacemakers. Thanks Todd, Amy, and Janet, without this donation we would not have been able to recruit such an esteemed researcher.

Inside this issue

Congenital Heart Walk Success!

We are pleased to announce that Georgia's 1st Congenital Heart Walk was a huge success! Hosted by and benefiting the missions of The Adult Congenital Heart Association and The Children's Heart Foundation, Atlanta's inaugural Walk raised over $77,000 for Congenital Heart Disease research. Over 70 teams united in the fight against Congenital Heart Disease, Saturday, May 10th at Stone Mountain Park with a mission of spreading CHD awareness. This premier event was sponsored by The Congenital Heart Center of Georgia (a collaboration between Emory and CHOA), Pediatric Cardiology Services, and several other local businesses. It was a day full of fellowship, dancing, great food, contests, prizes and a lot of family fun. Planning is currently underway for the 2015 Walk, and we are looking forward to making the Congenital Heart Walk an annual event. If you are interested in volunteering as a Walk planning committee member or would like additional information, please email: CHWGeorgia@gmail.com

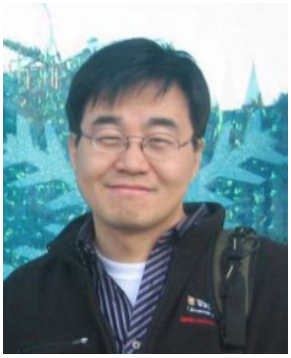
Tissue Engineering Approach Promotes Robust Enrichment of Cardiomyocytes Derived from Human Pluripotent Stem Cells



Nearly all cells from dissociated cardiospheres were cardiomyocytes according to immunocytochemical analysis with antibodies against a-actinin (green), a cardiomyocyte-associated marker, and DAPI (blue). For more details, please see Nguyen *et al.*, *Stem Cell Reports* doi:10.1016/j.stemcr.2014.06.002.

Cardiomyocytes (heart cells) derived from human pluripotent stem cells (hPSCs) are a promising cell source for regenerative medicine, disease modeling and drug discovery, which require enriched cardiomyocytes ideally with mature phenotypes. hPSCs have extensive proliferation capacity and have ability to differentiate into functional cardiomyocytes when cultured in specific conditions. However, current methods are typically performed in 2D environments that produce immature cardiomyocytes within heterogeneous populations. In a recent paper published in *Stem Cell Reports*, Xu, McDevitt and colleagues show that microscale formation of 3D constructs of cardiomyocytes, called cardiospheres, enables robust enrichment of cardiomyocytes from differentiation cultures, even when differentiation efficiency is low. They generated cardiospheres from 2D differentiation culture of hPSCs using a microscale technology and rotary orbital suspension culture. Nearly 100% of cardiospheres showed spontaneous beating and synchronous intracellular calcium transients, which are expected features for heart cells. Strikingly, ~80% to 100% cardiomyocytes were generated from starting heterogeneous cell populations. In addition, they performed cell morphology analysis and found that cardiomyocytes from cardiospheres exhibited enhanced structural maturation compared with those from parallel 2D culture. The technology developed in this study for enrichment of cardiomyocytes in microtissues has the potential for clinical and other applications.

Welcome Changwon Park, PhD



Please join us in welcoming Dr. Changwon Park, the Center's newest faculty member. Dr. Park comes to us from the University of Illinois at Chicago.

Heart disease accounts for 26% of deaths annually in the United States. More knowledge is needed about how the cardiovascular system develops and how it functions to devise better treatments for cardiovascular diseases. FLK1, a receptor tyrosine kinase, is essential for cardiovascular development and in the adult. It also plays a crucial role in neovascularization. Therefore, understanding molecular mechanisms regulating generation and function of $FLK1^+$ cells that give rise to blood vessels and blood cells is essential for delineating the pathways involved in differentiation of vessels as well as postnatal (cardio) vascular regeneration. However, the molecular mechanisms of $FLK1^+$ cell generation and function still remains unclear. Recently, we have demonstrated that the transcription factor ER71 (also known as ETV2) is a critical regulator of FLK1 and thereby it is essential for the cardiovascular system.

The long-term goal of my group is to understand the detailed mechanism of cardiovascular development and in postnatal (cardio) vascular regeneration with a special emphasis on transcriptional regulation including ER71/ETV2. To this end, we are currently employing mouse embryonic stem cell differentiation, mouse genetics, genome wide expression assay, NGS (next generation sequencing) including Chromatin Immunoprecipiation (ChIP)-sequencing and miRNA sequencing in conjunction with mouse injury models. The outcome of this work will significantly advance our knowledge on the role of key transcription factors not only in the cardiovascular system but also in neovascularization under pathophysiological conditions. In this regard, we are planning to identify small molecules or chemicals which can target key transcription factors for vascular regeneration via high-throughput screening. This could lead to the development of novel, effective therapeutic strategies for diseases related to dysfunctional vessel formation, an important translational aspect of the proposed studies.

In addition, my lab is interested in the generation of functional endothelial cells directly from somatic cells such as skin fibroblasts (direct reprogramming), aiming autologous cell replacement therapy.

2014 Pilot Project Awardees

Cardiac Fiber Imaging Using Ultrasound and DTI
Baowei Fei, PhD
Mary Wagner, PhD
Zhang Xiaodong, PhD

Impaired Collateral Vessel Formation in an Animal Model of Sickle Cell Disease
Robert W. Taylor, MD, PhD
David Archer, PhD

RBC Sorting by Microfluidic Chip: Understanding Biophysical Changes in Stores/Irradiated RBCs
Cassandra Josephson, MD
Wilbur Lam, MD, PhD
Nina Guzzetta, MD
John Roback, MD, PhD

MilliPub Club Induction

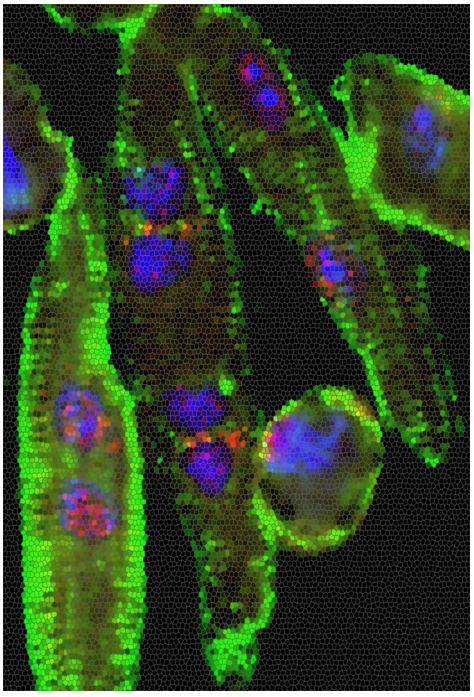
Congratulations Dr. Chunhui Xu on your iMilliPub Club induction. Dr. Xu's 2001 Nature Biotechnology paper " **Feeder-Free Growth of Undifferentiated Human Embryonic Stem Cells** " has reached +1,000 citations.

Regenerative Potential of the Young Heart

A multi-national collaborative team of researchers, funded in part from a pilot grant from the Center for Cardiovascular Biology, have reported a surprising finding that there is a short window of time in pre-adolescent mice in which heart growth is due to an increase in the number of heart cells, not just in heart cell size. Drs. Nawazish Naqvi and Ahsan Husain from adult cardiology at Emory, along with Dr. Mary Wagner from Pediatrics at Emory and colleagues from the Victor Chang Cardiac Research Institute in Sydney, Australia have published this exciting and novel finding in a recent issue of the journal *Cell.*

Dogma in the field of cardiology has been that the heart is a terminally differentiated organ, meaning that each person is born with all of the heart cells that they will ever have. This implied that the heart grows not by increasing the number of cardiomyocytes in the heart but by cardiomyocytes getting larger. Recent studies have shown that there is a short time of cardiomyocyte proliferation in the immediate postnatal period, but that cardiomyocytes withdraw from the cell cycle shortly after birth (postnatal day 7 in mouse (P7)). In their recent *Cell* paper, Dr. Naqvi *et al.* show that there is a second burst of cell proliferation in early adolescence, P15 in the mouse, increasing cardiomyocyte cell count by 40%. By meticulously examining cell number and histology of mouse hearts in the postnatal period, they found that a very short window of time (24 hours) in which myocytes reenter the cell cycle and divide, thereby increasing the number of cells in the heart. Cell growth then accounts for the continued increase in heart size as the mouse develops.

To determine possible mechanisms for this postnatal proliferative burst, the investigators measured serum thyroid hormone tri-iodothyronine (T3) levels during the postnatal period and discovered a large increase in T3 levels preceding increases in cell number. They then treated the mice with propylthiouracil (PTU) to inhibit synthesis of T3 which inhibited heart growth. They also showed that the T3 effect is likely mediated by insulin-like growth factor 1 (IGF1) pathway. Furthermore, in an exciting set of experiments, the investigators showed that there was increased regenerative potential during the postnatal proliferative burst. Using a myocardial infarction model, they confirmed that early neonatal mice (P2) were able to regenerate myocardium after injury and older mice (P21) were not. Furthermore, they showed, for the first time, that mice at P15 were also able to regenerate myocardium in response to injury, although not as well as in the very young mice. These findings suggest that there is an additional regenerative window for the heart in childhood, past infancy, which may have implications for treatment of children with congenital heart disease. Furthermore, understanding the mechanism by which myocytes in the postnatal heart reenter the cell cycle may identify new therapeutic targets for promoting cardiac regeneration and repair.



On the cover: Incredibly, the child's heart can expand many folds during preadolescence. Naqvi et al. show that, in the murine heart, this extreme growth is regulated by thyroid hormone, which activates cardiac insulin-like growth factor-1 signaling, and causes a replicative burst in mainly binuclear cardiomyocytes on a single developmental day during the third week of life. The image depicts immunocytochemical localization of the mitosis-promoting kinase aurora B (red), alpha-myosin heavy chain (green), and nuclei (blue) in both multinuclear and mononuclear cardiomyocytes. Note the disruption of sarcomeres in mature cardiomyocytes, as shown by a decrease in alpha-myosin heavy chain labeling, which is required to allow chromosome movement during mitosis. Illustration by Ahsan Husain.

Regenerative Potential of the Young Heart Continued

This significant discovery was highlighted by both scientific news reports as well as in the lay press. In addition, at the time of publication, this study was highlighted in the Leading Edge Previews of *Cell, by the journal Nature for its Research Highlights* This Week section and by an editorial Commentary in *Circulation Research.* The authors wish to thank the Center for Cardiovascular Biology for their support of this study.

Publication:

Naqvi N, Li M, Calvert JW, Tejada T, Lambert JP, Wu J, Kesteven SH, Holman SR, Matsuda T, Lovelock JD, Howard WW, Iismaa SE, Chan A, Crawford BH, **Wagner MB**, Martin DI, Lefer DJ, Graham RM, **Husain A**. (2014). A proliferative burst establishes the final cardiomyocyte population number during preadolescence. *Cell*, **157**, 795–807. PMID: 24813607.

Editorial Highlights:

Nathan J. Palpant NJ, and Murry CE. (2014). Leading Edge Previews. Proliferation at the heart of preadolescence. *Cell*. **157**, 765-767.

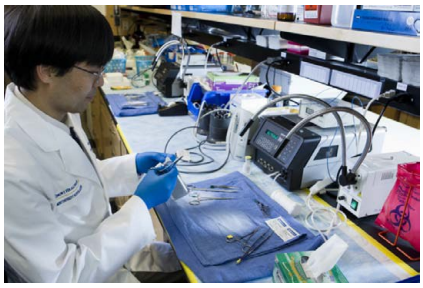
Research Highlights. (2014). Thyroid makes young hearts grow. Nature. 509, 263.

Zhang C-H, and Kuhn B. (2014). Editorial Commentary: Cutting-edge Research. Muscling up the heart: a preadolescent cardiomyocyte proliferation spurt contributes to heart growth. *Circ Res.* (in press).

Animal Physiology Core

The Animal Physiology (AP) Core is supported by the Emory+Children's Pediatric Research Center and is directed through the Center for Cardiovascular Biology. The AP core is a service center and offers acute and survival surgery to create disease models using small animals, such as rats and mice. Options include using our trained surgical staff to perform procedures or we will provide access to the surgical equipment for laboratories to perform their own procedures. We are located in ECC 260. Surgical procedures available include: Pulmonary artery or aortic banding to create heart failure, Myocardial infarction or cardiac ischemia/reperfusion, Liver ischemia/reperfusion, Tail vein injections, and Renal failure by 2 step nephrectomy. In addition, we are happy to assist with the development of additional disease models. Equipment available includes: Small animal ventilator, Cautery, Temperature monitoring, Pulse oximeter, Isoflurane anesthesia system, and Dissecting microscope with fluorescence

The AP core also offers high-resolution (30 µm) *in vivo* imaging ideally suited for small animals such as mice and rats. Ultrasound examinations can be used to characterize cardiac function and liver and kidney blood flow. Studies can either be conducted for you by our trained staff or investigators can reserve the equipment and utilize their own laboratory personnel. Core staff are currently trained to do ultrasound examinations for cardiac function and liver and kidney blood flow. We will work with investigators to optimize their experiments. Equipment and modalities include: Visualsonics Vevo 2100 Ultrasound System, Integrated isoflurane -based anesthesia system, B-Mode and M-Mode cardiac imaging modalities, Pulsed-Wave and Color Doppler Modes. Contrast imaging functionality, and strain imaging for cardiac mechanic measurements.



Animal Physiology Core

Emory Children's Center
Room 260

For questions, please contact:
Mary Wagner, Scientific Director
mary.wagner@emory.edu

Subsidies are available to pediatric researchers.


Drs. Nina Guzzetta and Tom Barker

Utilizing Platelet-Like Particles Following Neonatal Cardiac Surgery

This project utilizes innovative platelet-like particles (PLPs), recently designed in Dr. Barker's lab, to develop a novel hemostatic agent for use in the clinical arena of coagulopathy after congenital cardiac surgery. Our team is comprised of clinicians, engineers and basic scientists that are uniquely suited to investigate this problem. Drs. Guzzetta, Barker, Lyon and Lam are recognized experts in management of bleeding during neonatal CPB, fibrin/fibrin-based biomaterials, hydrogels/colloids and platelet contraction/biophysics and microfluidics, respectively. We plan to take advantage of these multidisciplinary skill sets in order to successfully develop technology intended to dramatically lessen the transfusion requirements of neonates undergoing cardiac surgery and CPB.

The overarching goal of this project is to create a biomaterial that recapitulates key hemostatic functions of platelets to augment clotting in neonatal cardiac surgery patients after CPB. To achieve this objective, we will utilize platelet-like particles (PLPs), recently designed in Dr. Barker's lab, that mimic numerous features of natural platelets by interacting with fibrin with high affinity and, more importantly, specificity at the sites of injury to augment clotting in plasma samples obtained from neonates undergoing CPB. To achieve our objective, the proposal is divided into two specific aims: 1. Characterization of platelet function and clotting in neonatal CPB plasma samples in the a) absence and b) presence of PLPs and 2. Characterization of augmentation of clotting in vivo in a rodent model of platelet hyporeactivity.

The proposed research is innovative because it combines unique microgels with fibrin specific binding motifs to create PLPs that interact extensively with fibrin networks. These features result in particles that are capable of recapitulating more features of natural platelets than previously achieved by other platelet-mimicking materials. The significance of the proposed research is that this work will enable better treatment options for coagulopathy in neonatal cardiac surgery patients after CPB.

Having completed a substantial amount of the above work, we are pleased to share that results have led to a NIH funded grant for $275,000 over two years. In addition, we recently published a paper within the prestigious journal *Nature Materials*. Future submissions are in progress for this innovative and productive collaboration.

Trends in Funding Proposals

CCB, in collaboration with the Sibley Heart Center, continues to establish a productive trend as it relates to extramural funding proposals. The current fiscal year demonstrates a combined submission of more than $4 million. As our collaborations grow, in conjunction with the growth of Center faculty and members, we anticipate continuing to share this upward trajectory.

Center members, please remember to site the Center on funding proposals as well as publications. For additional details, please click here.