The XXV Annual Meeting
Israeli Group for Heart Research
Subsection of the ISHR - European Section

Felsenstein Medical Research Center
Beilinson Campus, Tel Aviv University

February 23, 2009

Organizing Committee

Edith Hochhauser, Gan-ia Kessler-Icekson, Michael Arad

International Society for Heart Research
European Section
ISRAELI SUBSECTION

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The XXV Annual Conference of the Israeli Subsection of the International Society for Heart Research – European Section

Felsenstein Medical Research Center, Beilinson Campus, Tel Aviv University, February 23, 2009

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Program

08:30-09:00 Registration

09:00-09:15 **Greetings**
Avi Weizman Director, *Felsenstein Medical Research Center*
Eyran Halpern C.E.O., *Rabin Medical Center*
Boaz Tadmor Director, *Beilinson Hospital, Rabin Medical Center*
Yosef A. Mekori Dean, *Sackler Faculty of Medicine, Tel Aviv University*

09:15-10:00 **Keynote Lecture**: ***Professor Nader G. Abraham***
Director of Gene Therapy Program, New York Medical College,Valhalla, NY, U.S.A
Distinct regulation of inflammatory cytokines and adiponectin by HO-1 induction leads to amelioration of diabetes and adiposity.

10:00-11:30 **Session I - The Rena Yarom Young Investgator Competition**
Moderators: Arie Moran, Uri Oron and Hertzl Schwalb

10:00-10:15 *The transcription Factor Islet-1: A novel gene target for future cardiac repair?* Aya Barzelay, The Cardiology Department, Tel Aviv Sourasky Medical Center and Felsenstein Medical Research Center, Tel Aviv University, Petach Tikva

10:15-10:30 *Macrophages are essential for infarct repair with and without stem cell therapy* Tammy Ben-Mordechai, Neufeld Cardiac Research Institute, Tel Aviv University, Tel Aviv

10:30-10:45 *Transgenic model for cardiac remodeling: insights and applications* Gordon Oren, Departments Molecular Biology and Cardiology, The Hebrew University -Hadassah University Hospital, Jerusalem

10:45-11:00 *Quantification of cross-bridge cycling between the different physicochemical conformations by optical means.* Tamar Harary, Faculty of Biomedical Engineering, Technion, Israel Institute of Technology, Haifa

11:00-11:15 *Optimizing CPVT therapy in calsequestrin-mutant mice* Guy Katz, The Heart Institute, Sheba Medical Center, Tel Hashomer, Ramat Gan

11:15-11:30 *A histone deacetylase inhibitory prodrug attenuates doxorubicin-cardiotoxicity while augmenting doxorubicin anticancer activity* Nataly Tarasenko, Felsenstein Medical Research Center, Tel Aviv University, Petach Tikva

11:30-11:45 **ISHR Business Meeting**

11:45 -12:15 **Lunch Break**

12:15-13:45 **Session II- Heart Development; Cell Therapy**
***Moderators*:** Gan-ia Kessler Icekson and Amir Landesberg

12:15-12:30 *Early embryonic regulation of the cardiac field in chick embryos* Miriam Ivenshitz, Department of Biological Regulation, Weizmann Institute of Science, Rehovot

12:30-12:45 *Spatiotemporal inhibition of Fgf signaling by BMP4 promotes splanchnic mesoderm differentiation and myofibrillogenesis* Libbat Tirosh-Finkel Department of Biological Regulation, Weizmann Institute of Science, Rehovot

12:45-13:00 *Isolation of c-kit positive cardiac progenitor cells from the human heart: Origin of cells and correlation with patient characteristics* Ayelet Itzhaki-Alfia, Neufeld Cardiac Research Institute, Sackler Faculty of Medicine, Tel-Aviv University, Sheba Medical Center, Tel-Hashomer, Ramat Gan

13:00-13:15 *Fibrinogen C-terminal sequences (Haptides) cause a dramatic decrease in blood pressure in rats* Maamoun Basheer, Laboratory of Biotechnology and Radiobiology, Sharett Institute of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem

13:15-13:30 *Hybrid therapy of alginate biomaterial injection and staged cardiomyocyte transplantation improves infarct healing and cardiac remodeling in rat* Natalie Landa, Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer, Ramat Gan

13:30-13:45 *Hypoxia Inducible factor-alpha improves the migratory properties of bone-marrow derived mesenchymal cells* Jonathan Semo, Department of Cardiology, Tel-Aviv Sourasky Medical Center, Tel Aviv

13:45-14:00 **Coffee break**

14:00-15:45 **Session III - Arrhythmia and Hypoxia**
***Moderators*:** Michael Arad and Edith Hochhauser

14:00-14:30 **Special lecture *Ronny Alcalai***, Department of Cardiology, Hadassah Hospital, Hebrew University, Jerusalem
LAMP2 Cardiomyopathy: The Consequence of Impaired Autophagy

14:30-14:45 *ZnT-1, a novel regulator of T- type calcium channels mediating a crosstalk between T-type and L-type calcium channels* Merav Mor, Physiology Department Faculty of Health Sciences Ben Gurion University, Beer Sheva,

14:45-15:00 *Abbreviated effective refractory period and amplified dispersion of repolarization underlie the development of atrial fibrillation in a canine atrial wedge model of short QT1: Mechanism and implications for anti arrhythmic therapy* Eyal Nof, The Heart Institute, Chaim Sheba Medical Center, Ramat Gan

15:00-15:15 *Autophagy is required for preconditioning by the Adenosine A1 receptor-selective agonist CCPA* Smadar Yitzhaki, BioScience Center, San Diego State University, San Diego, CA, USA

15:15-15:30 *Rapamycin protects heart cultures against Hypoxia via inhibition of SERCA2A and activation of PKC and MAPK* Dalia El-Ani, Heart Institute, Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Tel Aviv

15:30-15:45 *Physiological and molecular evidence of heat acclimation mediated cross-tolerance memory: a lesson from the heart.* Anna Tetievsky, The Hebrew University, Jerusalem, Israel

15:45-16:00 **Coffee Break**

16:00-17:30 **Session IV - Blood Vessels and Heart Muscle Function**
***Moderators*:** Asher Shainberg and Michal Horowitz

16:00-16:15 *Flow induction of blood-vessel network within engineered tissues* Ayelet Lesman, Biomedical Engineering, Technion- Israel Institute of Technology, Haifa

16:15-16:30 *Plaque neovascularization and its quantification* Assaf Hoogi, Technion, Haifa

16:30-16:45 *A functional role for eotaxin-2 in the initiation and progression of experimental atheroma* Michal Entin-Meer, Department of Cardiology, Tel-Aviv Sourasky Medical Center, The Sackler Faculty of Medicine, Tel Aviv

16:45-17:00 *Vessel/Myocardium interaction affecting intramyocardial dynamic flow* Dotan Algranati, Faculty of Biomedical Engineering, Technion, Haifa

17:00-17:15 *The external-work pressure-time integral relationships and the afterload dependence of frank starling mechanism* Gali Sela, The Faculty of Biomedical Engineering, The Technion, Haifa

17:15-17:30 **Rena Yarom Award, Concluding Remarks**

The transcription Factor Islet-1: A novel gene target for future cardiac repair?

Aya Barzelay [1,2] , *Edith Hochhauser*[2,3],*Arnon Afek*[4], *Yelena Chepurko*[3], *Einat Birk* [2,5], *Lidya Pinhas*[5] , *Michal Entin – Meer*[1], *Sofia Maysel- Auslender*[1] , *Gad Keren*[1,2], *Jacob George*[1,2].

[1] The Cardiology Department, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[2] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[3]The Cardiac Research Laboratory, Felsenstein Medical Research Center, Rabin Medical Center, Tel Aviv University, Petah Tikva, Israel
[4]Institute of Pathology, Sheba Medical Center, Tel Hashomer, Israel
[5] Department of Cardiology, Schneider Children's Medical Center, Petah Tiqva Israel

The LIM-homeobox transcription factor isl1 plays a crucial role during heart embryogenesis. Embryonic isl1+ precursors give rise to over two-thirds of the heart and to its subsequent lineages: cardiac muscle, smooth muscle and endothelium. Interestingly, a subset of Isl1+ progenitors remains embedded in the postnatal heart.
In the current study, we investigated whether isl1 is expressed in *adult* mesenchymal stem cells (MSCs), and after acute myocardial infarction (MI). Additionally, we examined whether isl1 overexpression in MSCs could promote their vasculogenic properties, and whether intramyocardial gene transfer of naked DNA encoding isl1 could promote a functional recovery after MI.
We used the transgenic mice *isl1/cre/Z/EG*, in order to detect isl1 expression in MSCs of adult mice, complemented by qRT-PCR and immunostaining for isl1 detection in rat- MSCs. Four weeks after MI induction, isl1 expression was assessed in bone marrow and spleen cells by qRT-PCR and immunostaining. Isl1 was retrovirally transduced to MSCs. endothelial markers were examined by FACS and tube formation capacity was assessed on matrigel. Furthermore, intramyocardial injection of plasmid encoding isl1 to mice after ligation of the LAD has been performed.
We report for the first time, the identification of isl1+ progenitors in *adult* bone marrow. The number of isl1+ progenitors increased after *in vitro* cell culture, and also in the splenocytes after acute experimental myocardial infarction. Isl1 overexpression in MSCs promoted their differentiation towards endothelium. Finally, isl1 gene transfer to the peri-infarct region after MI induction resulted in partial salvage of ventricular function evident by echocardiography.
Thus, the isl1 gene appears as an attractive target for future cell based therapy for regenerative myocardial dysfunction.

Macrophages are essential for infarct repair with and without stem cell therapy

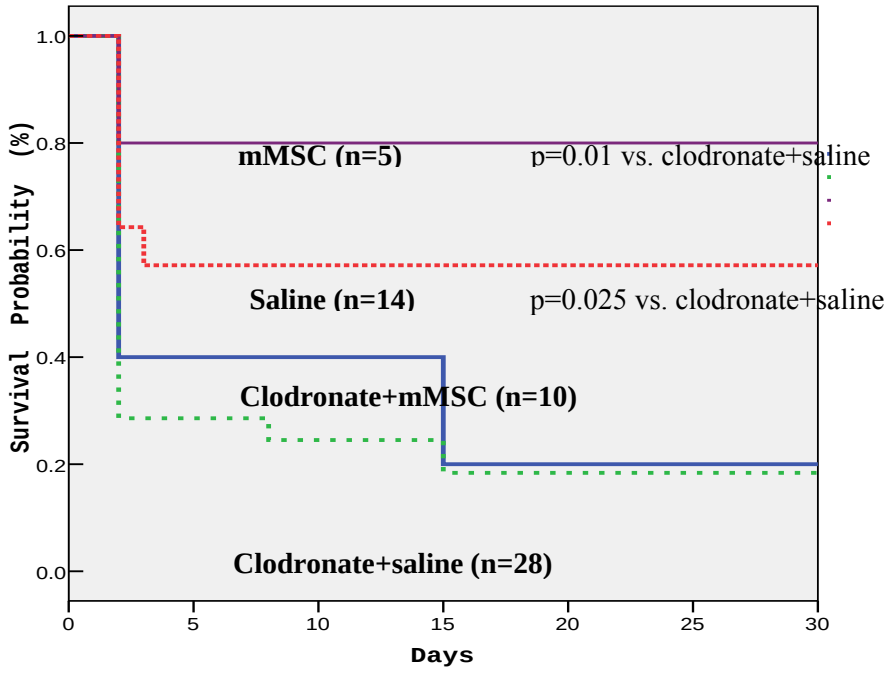
Tammy Ben-Mordechai[1], *Radka Holbova*[1], *Adi Zuloff-Shani*[2], *Micha S. Feinberg*[1], *Zmira Silman*[1], *David Danon*[2], *Jonathan Leor*[1].
Neufeld Cardiac Research Institute, Tel Aviv University[1]. Research and Development Unit, Blood Service Center, Magen David Adom[2].

Myocardial healing is impaired in elderly and sick people partially because of defective inflammatory response. The present study aimed to determine the significance of macrophage (MΦ) activity in infarct repair and to test the hypothesis that young MΦ can improve infarct repair in the aged mouse.

Methods and Results:

MΦ depletion was induced by clodronate injection (IV or IP) to Balb/C mice. Animals (n=57) with and without MΦ depletion were subjected to MI and randomized to injection of mesenchymal stem cells (mMSC; n=15) into the infarct or saline (n=42). Mortality after MI was significantly higher in MΦ depleted mice, with and without stem cell therapy, compared with controls (Figure1; p=0.02).

Figure 1: Survival curves of the four study groups**.**



In the next experiment, MΦ were isolated from the peritoneum of young (12w) or aged (8m) Balb/c mouse. The young or old MΦs (50,000) were injected into the infarcted myocardium of aged (8 months) mice (n=9, n=8; respectively) immediately after MI. The control group (n=9) was treated with saline injection. Serial echocardiography studies were performed 1 day and 4 weeks after MI. After 4 weeks, aged animals treated with saline or old MΦ experienced significant increase in infarct thinning and LV dilatation ($p<0.02$), while this variables of adverse remodeling were attenuated in animals treated with young MΦ.

Conclusions:

Macrophages are essential for infarct repair with and without stem cell therapy. The administration of young macrophages to repair MI could be important to sick and elderly people in whom the availability of autologous, functional stem cells is limited.

Transgenic model for cardiac remodeling: insights and applications

Gordon Oren, Dan Gilon and Eli Keshet
Departments of Molecular Biology and Cardiology, The Hebrew University-Hadassah University Hospital, Jerusalem , Israel.

Background: Ventricular remodeling describes structural changes in the left ventricle in response to myocardial injury, including hypertrophy, stretching and ventricular dilatation. Accompanying alterations in the interstitial extracellular matrix represent an adaptive response aiding the thinned ventricular wall to withstand increased pressure. However, traditionally considered a beneficial mechanism, remodeling often become maladaptive and a contributor to an eventual heart failure. Accordingly, reversing maladaptive remodeling has been recognized as a prime goal in heart failure prevention and remodeling-reversing drugs are already in routine use. Yet, molecular mediators and signaling pathways governing transition from adaptive to maladaptive remodeling are poorly understood. To uncouple a remodeling response from the amalgam of other processes associated with myocardial infarction, we have developed a unique mouse transgenic system for conditional infliction of cardiac insufficiency inducing, in turn, LV remodeling. Importantly, our system allows to fully reverse remodeling upon restoration of normal cardiac function or through administration of remodeling-reversing drugs. **Aims:** Using this unique experimental platform, we aim at defining the different stages in the development of LV remodeling, elucidating the remodeling genetic program through high-throughput analyses and to offer this system as a platform for testing new remodeling-reversing drugs. **Methods:** Our system is based on the soluble VEGF receptor 1, which acts as a decoy receptor that binds VEGF and blocks its activity. Induction of sVEGFR-1 post natal, when the mouse heart is fully developed but still grows is size, leads to a formation of a hypo-vascular and hypoxic heart. Following sVEGFR-1 withdrawal hypoxia is relieved by re-vascularization. **Results:** In our model, the heart is capable of reducing hypoxia levels by stopping contraction of viable cardiomyocytes without cell death, creating myocardial hibernation. Consequently, LV remodeling is developed. Notably, this process occurs gradually. During the initial phase hypoxia is high and various K^+ channels related genes are upregulated. These may serve to reduce the contractility of cardiomyocytes. As a result myocardial demand is lowered and hypoxia is reduced. Now, Extra Cellular Matrix (ECM) genes are expressed enabling the myocardium to endure the continued work load. These changes are first manifested as interstitial fibrosis accompanying left ventricle dilatation and left ventricular hypertrophy develops only later. We propose utilizing this system as a platform for testing new anti-remodeling drugs. As a proof of principle, we have tested Aliskiren, a new direct renin inhibitor recently developed. We show that Aliskiren is capable of reversing the remodeling process as well as, if not better than, the ACE inhibitor Enalapril. **Conclusions:** We developed a new mouse transgenic system for the induction and reversal of cardiac remodeling. We used this model to explore the basic pathophysiology of LV remodeling and found that following sub-acute ischemia ventricular dilatation and fibrosis precede ventricular hypertrophy. Using high-throughput analyses we defined the genetic program of cardiac remodeling. We propose this system as a platform to test new remodeling-reversing drugs. As proof of principle we show that both ACE inhibitors and direct Renin inhibitors are capable of reversing cardiac remodeling in our model, roughly in the same efficiency.

Quantification of cross-bridge cycling between the different physicochemical conformations by optical means.

Tamar Harary, Amir Landesberg.
Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel

Introduction: When the linearly polarized light enters the optically anisotropic muscle fiber the transmitted light obtains an elliptical polarization. The transmitted light polarization is determined by the sarcomere alignment, the size of the filament overlap zone and the changes in the cross-bridge (XB) distribution between the various physicochemical conformations.
Aims: To quantify the changes in cardiac muscle optical properties during contraction and to differentiate between the effects of the sarcomere length (SL) and those of XB cycling. To explore the relationships between the changes in the optical properties and the dynamics of XB recruitment.
Methods: Thin trabeculae (n=6) were isolated from rat right ventricles (K-H solution, 25°C). SL was measured by laser diffraction technique and controlled by a fast servomotor. The polarized HeNe laser spot was reduced to less than 90μm by a beam expender. The direction of the incident light polarization was set to 45° relative to the fiber axis by a half wave length plate. The transmitted light passes though liner polarizer towards the photo detector. The changes in the transmitted light polarization were measured at all the directions, during rest, twitch contraction, sarcomere control isometric contractions (1.97μm), and at different calcium concentrations (0.75, 1.5, 4.5 [mM]).
Results: The time to peak change in the transmitted light intensity preceded ($50 \pm 10 msec$) the peak force (120msec), and the transition back to baseline intensity lagged behind force relaxation. There was a tight correlation between the rate of changes in the light intensity and the rate of force development. The degree of polarization decreased during force development, reaching a minimal value close to the peak force, and it increased back towards baseline during the relaxation phase. These observations could not be attributed to changes in the SL since similar phenomena were observed during isometric sarcomere contractions, and the variations in the degree of polarization were even greater (13%) during sarcomere isomeric contractions. The role of the XBs was furtherer validated by imposing isometric contractions at the same SL but different force levels with different extra-cellular calcium concentrations. Higher calcium level and larger force yielded a larger shift in the polarization at the same SL.
Significance: Optical measurements provide additional information about XB dynamics that differ from the observed dynamics of force or stiffness measurements, and it can be used for quantifying cardiac muscle activation and XB cycling.

Optimizing CPVT therapy in calsequestrin-mutant mice

Guy Katz[1], *Efrat Kurtzwald*[1,2], *Edith Hochauser*[2], *Yelena Chepurko*[2], *Eyal Porat*[2], *Asher Shainberg*[3], *Jonathan G. Seidman*[4], *Michael Eldar*[1], *and Michael Arad*[1]
[1]Heart Institute, Sheba Medical Center, Tel Hashomer
[2]Cardiac Research Lab, Felsenstein Medical Research Center, Sackler School of Medicine, Tel Aviv University
[3]Department of Life Sciences, Bar Ilan University, Israel
[4]Department of Genetics, Harvard Medical School, Boston, USA

Background Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal human arrhythmia provoked by physical or emotional stress and mediated by spontaneous Ca^{++} release and delayed after-depolarizations. Beta-adrenergic blockers are the therapy of choice for human CPVT, but achieve complete arrhythmia control in,<50% of cases. Using a murine model of recessively-inherited CPVT caused by either a D307H mutation ($CASQ2^{D307H}$) or CASQ2 knock-out ($CASQ2^{\Delta}$), we conducted a pharmacological screen to optimize the medical therapy of CPVT.
Methods Heart rhythm telemetry was obtained in awake animals at rest, during treadmill exercise and after intra-peritoneal (IP) injection of epinephrine [0.5μg/g]. The protocol was repeated after IP injection of different anti-arrhythmic agents. The primary end-point was the ability to induce CPVT, defined as VT recorded in an animal during any one of the stress protocol stages.
Results Adult CASQ2 mutant mice suffered from complex ventricular arrhythmia at rest and developed bidirectional and polymorphic VT on exertion. Class I antiarrhythmic agents (procainamide, lidocaine, flecainide) were ineffective in controlling arrhythmia. Propranolol and sotalol attenuated arrhythmia at rest but failed to prevent CPVT during sympathetic stimulation. The calcium channel blocker verapamil showed a dose-dependent and genotype-dependent protection against CPVT. Verapamil was more effective than the dihydropyridine L-type Ca^{++}-channel blocker nifedipine, and its activity was markedly enhanced when combined with propranolol. Experimental agents; nicorandil (opening the ATP-dependent K channel), Cl-IB-MECA (an A3 adenosine agonist increasing SERCA activity) and a novel forward-mode NCX inhibitor did not affect arrhythmia prevalence or its severity.
Conclusions High dose verapamil is the antiarrhythmic drug of choice in CASQ2 mice. L-type Ca^{++} channel blockade is only one of the mechanisms participating in CPVT suppression by verapamil. Beta-adrenergic blockers have little benefit in murine CPVT but markedly enhance the effects of verapamil.

A Histone deacetylase inhibitory prodrug attenuates doxorubicin-carditoxicity while augmenting doxorubicin anticancer activity

Nataly Tarasenko,[1] *Gania Kessler-Icekson,*[1] *Abraham Nudelman,*[2] *Hadassa Schlesinger,*[1] *Ada Rephaeli*[1]
[1]Felsenstein Medical Research Center, Tel Aviv University, Petach Tikva
[2]Chemistry Department, Bar-Ilan University, Ramat-Gan, Israel

Background Cardiotoxicity limits the clinical use of doxorubicin (Dox) as an anticancer agent, largely due to Dox-induced reactive oxygen species (ROS). Previously, we have shown that the HDAC inhibitory prodrug butyroyloxymethyl diethylphosphate (AN-7) protects cardiomyocytes and the heart of naïve mice from Dox-induced toxicity. Moreover, AN-7 interacted synergistically with Dox to induce mortality of cancer cells in vitro.
The purpose of this study was to investigate whether the opposing effects of AN-7 and Dox combination treatment observed in vitro, will be preserved in tumor bearing mice, and to characterize molecular changes associated with these activities in their hearts and tumors.
Methods The effects of Dox, AN-7, and their combination, were tested in vitro on neonatal rat cardiomyocytes and 4T1 murine breast carcinoma cell line, and in vivo on a syngeneic metastatic 4T1 murine model**.** The treatment associated changes in the hearts and the tumors were evaluated using Western blot analyses and immunohistochemistry (IHC).
Results In cardiomyocytes, AN-7 attenuated Dox toxicity and abrogated Dox-provoked ROS formation, whereas in 4T1 cells, AN-7 and Dox induced a synergistic ROS-dependent cell death. In mice bearing 4T1 tumors, treatment with the combination of Dox and AN-7 prevented Dox-induced increase in the heart weight-to-body weight ratio, reduced mortality caused by Dox, and significantly reduced tumor weight and the number of lung metastases. In the heart and the tumors of the same mice opposing effects of the combination treatments were observed. In the hearts, increased levels of heme oxigenase-1 (cyto-protective), bFGF (pro-angiogenic), and c-Myc protein (transcription regulator) were detected. In Dox-treated hearts, the levels of pH2AX (a marker of dsDNA breaks) and TSP1 (anti-angiogenic), were elevated whereas a significant decrease in these markers was found in mice treated with Dox and AN-7, indicating that AN-7 imparted cardioprotection. In the tumors, treatment with AN-7 or Dox, reduced the levels of c-Myc and elevated pH2AX, indicators of survival and DNA damage, respectively, and induced the appearance of cytoplasmic cytochrome-c, and the diminution of Ki67, markers of apoptosis and proliferation, respectively. The combination treatment markedly augmented these changes indicating increased anti-cancer activity.
Conclusions The combination treatment of AN-7 and Dox attenuates Dox-cardiotoxicity while potentiating Dox-anticancer activity. The dichotomy displayed by this adjuvant treatment in the heart versus tumor overcomes the major obstacle in achieving maximal efficacy of Dox-anticancer therapy and underscores the potential advantages of HDAC inhibitory prodrugs for cancer treatment.

Early embryonic regulation of the cardiac field in chick embryos

Miriam Ivenshitz and Eldad Tzahor
Department of Biological Regulation, Weizmann Institute of Science, Rehovot

In the developing embryo, cardiogenesis is a tightly controlled process, and is subject to strict temporal and spatial regulation. During early embryogenesis, heart progenitor cells take up residence in the bilateral anterior mesoderm and form the cardiac crescent. Signals from bordering endoderm and axial tissues act to promote or repress cardiogenesis, respectively. While the heart-inducing activity of the endoderm has been attributed to Bmp, Wnt antagonists, and Fgf8 signaling molecules, the molecular signals that limit early cardiogenesis to the lateral plate mesoderm perimeter and prevent it from invading into the medial mesoderm are unknown. We have shown that stage 6 anterior lateral (AL) plate mesoderm explants cultured for 24-48 hours spontaneously beat and display a repertoire of typical cardiac genes. In contrast, the anterior medial (AM) explants, which are normally fated to be head mesoderm, fail to beat and express no cardiac marker genes. Upon BMP4 application, AM explants were induced to undergo cardiogenesis, upregulate cardiac gene expression and spontaneously beat. On the other hand, AM explants which contain a small portion of the neural tube (AMNT) did not exhibit the same cardiogenic potential upon BMP4 administration, suggesting that there is another, BMP-independent, repressing signal from the neural tube. Further attempts, both in vitro and in vivo, to identify the source of this cardiac-inhibitory signal revealed that the repressive capability of the neural tube was located in the ventral neural tube as opposed to dorsal part. Ventral and dorsal neural tube (NT) tissues of quail origin were transplanted into the AL mesoderm of host chick embryos at identical stages, and embryos were left to develop until stage 8. Ventral NT, but not dorsal NT transplantation into AL mesoderm inhibited cardiogenesis, as seen by a dramatic decrease in the cardiac gene *Nkx2.5*. These results further indicate that there is a repressive signal(s) secreted from the ventral neural tube/notochord at cardiac crescent stages. We aim to identify and characterize this repressive signal involved in cardiogenesis control and regulation.

Spatiotemporal inhibition of Fgf signaling by BMP4 promotes splanchnic mesoderm differentiation and myofibrillogenesis

Libbat Tirosh-Finkel, Amit Zeisel[*]*, Eytan Domany*[*] *and Eldad Tzahor*
Department of Biological Regulation, [*] Department of Physics of Complex Systems
Weizmann Institute of Science, Rehovot 76100, Israel

Abstract: Our lab employs both *in vitro* and *in vivo* experimental systems in the avian embryo to explore how mesoderm progenitors in the head differentiate into both heart and skeletal muscles. The head mesoderm can be regionalized into two separate populations: the cranial paraxial mesoderm and the splanchnic mesoderm which, in culture, undergo myogenesis and cardiogenesis, respectively. We have shown that Bmp signaling affects the specification of mesoderm cells in the head: application of BMP4, both *in vitro* and *in vivo*, induces cardiac differentiation in the cranial paraxial mesoderm and blocks the differentiation of skeletal muscle precursors in these cells. The splanchnic mesoderm (SpM) encompassing the anterior heart field contributes to significant portions of the heart, including the outflow tract, the right ventricle and atria. These cells express distinct cardiac markers when grown in culture, yet rarely undergo final differentiation into beating cardiomyocytes. Administration of BMP4 induced beating of SpM-derived cardiomyocytes, suggesting a positive role for Bmp signaling in the terminal differentiation of cardiac progenitors. A large-scale microarray screen revealed novel insights on the role of the Bmp signaling pathway in the differentiation of cardiomyocytes; BMP4 markedly elevated the expression of numerous sarcomeric genes. In addition, we uncovered a tight crosstalk between the Bmp and Fgf signaling pathways in the SpM: BMP4 blocks Fgf signaling. Our findings indicate that BMP4 administration or inhibition of Fgf signaling (via either the MEK/ERK or the JUN pathways) dramatically reduced cell proliferation and enhanced myofibrillogenesis *in vitro*. We suggest that Bmp and Fgf signaling pathways are under tight spatiotemporal regulation, via their synexpression group members, to allow anterior heart field cells to differentiate at the right time and place.

Isolation of c-kit positive cardiac progenitor cells from the human heart: Origin of cells and correlation with patient characteristics

Ayelet Itzhaki-Alfia, Jonathan Leor, Ehud Raanani , Leonid Sternik , Dan Spiegelstein , Radka Holbova, Jacob Lavee, Israel M Barbash
Neufeld Cardiac Research Institute, Sackler Faculty of Medicine, Tel-Aviv University, Sheba Medical Center, Tel-Hashomer, Israel.

Background: The isolation and expansion of human cardiac stem cells (hCSCs) offers new therapies for myocardial regeneration and repair. The cells could be expanded in vitro providing large number of hCSCs for therapeutic applications. However, despite significant advance and promising findings in animals, several issues have not been profoundly investigated. First, an efficient approach is needed to isolate human cardiac progenitor cells (hCPCs) from human heart. Current methods are derived mostly from rodents and with variable efficiency, thereby limiting the potential clinical application of hCPCs. Second, hCPCs can be isolated from different sections of the heart, such as the septum and atrial appendages. However, correlation between the origin of the sample and the number of hCPCs has not yet been reported. Finally, while it has been reported that the number of hCPCs increase in heart diseases, it is unclear whether hCPCs could be isolated and grow from all patients.
Using a novel method to isolate hCPCs, we aimed to define the optimal source and association between hCPC number and patient characteristics.
Objective: Understanding the characteristics of hCPCs would be important in order to gain a better insight of their role in maintaining cardiac function and their therapeutic potential as cardiac progenitors for myocardial repair. To define the best cardiac source for these hCPCs, and to determine a possible association between patient characteristics and hCPC number.
Methods and Results: We developed a novel isolation protocol that enumerated viable cells (7x106 ±6.53x105 per gram), from various tissue samples obtained during heart surgery or endomyocardial biopsies (n=113; 94 patients aged 23-80 years). By FACS analysis, cultures derived from the right atrium generated higher amounts of C-kit+ (24±2.5%) and Isl-1+ cells (7%) compared with other locations (7.3±3.5% left atrium, 4.1±1.6% right ventricle and 9.7±3% left ventricle (LV); p=0.001). After adjusting for clinical parameters, female gender, impaired LV function and right atrium as a source of cells were associated with higher number of c-kit+ cells. In vitro assays of differentiation into osteoblasts, adipocytes, and myogenic lineage indicated that the isolated hCPCs were multipotent. Finally, the cells were transplanted into infarcted myocardium of rats and generated myocardial graft after one month.
Conclusions: The main findings of the present study suggest that it is feasible to isolate c-kit or islt-1 hCPCs from most patients undergoing open heart surgery, using the right atrium as the best source for c-kit hCPCs and higher amounts of c-kit+ cells could be produced from tissue samples obtained from females and patients with impaired LV function. In addition, here we present a novel, efficient and reproducible protocol to isolate ckit+ hCPCs from a diverse tissue samples. Our findings could enhance the development of novel approaches in regenerative cardiovascular medicine. Further research is needed to determine whether those cells could be used for replacement of injured myocardium by direct cell transplantation or in situ stimulation.

Fibrinogen C-terminal sequences (Haptides) cause a dramatic decrease in blood pressure in rats

Maamoun Basheer[1,2], *Herzl Schwalb*[2], *Maoz Nesher*[3], *Oz M Shapira*[2], *Dan Gilon*[4], *and Raphael Gorodetsky*[1]

[1]Laboratory of Biotechnology and Radiobiology, Sharett Institute of Oncology, [2]Cardiothoracic and Thoracic Research Center, Department of Cardiothoracic Surgery
[3]Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem Israel.
[4]Department of Cardiology Hadassah-Hebrew University Medical Center.

Background: Our previous studies indicated that Haptides synthetic conserved 19-21mer sequences homologous to the C-termini of fibrinogen chains β and γ (Haptides Cβ and preCγ, respectively) at levels of 30-100μg/ml induce transient severe coronary arteries vasoconstriction in isolated perfused hearts. This is accompanied by temporary decrease in myocardial function that possibly stem from Haptides induced reduction of NO levels.

Aim of the study: In-vivo assessment of the effect of intravenously administered Haptides in rats.

Methods and Results: Intravenous Haptides administration at lower concentrations (35-560 μg/kg rat) showed a dose dependent dramatic transient decrease in the systolic and diastolic blood pressures by up to 55% accompanied with increased heart rate by up to 7%. Arbitrarily scrambled sequences of the Haptides had no such effect, suggesting a specific receptor mediated effect. In the non-sedated rats intravenous administration of Haptides seemed to cause reduced mobility of the animals with a shock-like behavior. Intravenous administration of anti-histamine receptors pyrilamine and Cimetidine directed to receptors H1 and H2, respectively, 10 min prior to the Haptides administration, attenuated the decrease of systolic and diastolic blood pressures. These results suggest the involvement of histamine receptors in the blood pressure lowering mechanism. Moreover, nedocromil sodium, a mast cell stabilizer, partially attenuated the Haptides induced blood pressure decrease. In an in vitro experiment, incubation of mouse bone marrow derived mast cells with Haptides caused degranulation of the mast cells.

Conclusions: Our data suggest that Haptides Cβ and preCγ may activate mast cells, resulting with histamine release. This, in turn, may cause a steep decrease in blood pressure comparable to anaphylactic shock. Therefore, it is suggested that anti histaminic treatment in pathological condition such as vascular occlusive diseases, where the intensive degradation of fibrin takes place, may overcome this effect.

Hybrid therapy of alginate biomaterial injection and staged cardiomyocyte transplantation improves infarct healing and cardiac remodeling in rat

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Background: Cell transplantation into the infarct may be of limited benefit when the extracellular matrix (ECM) is damaged and cannot support cell retention. The aim of our study was to test the hypothesis that hybrid strategy combining injection of alginate biomaterial that replaces the damaged ECM followed by staged cell transplantation is superior to single therapy.
Methods and Results: We used a novel injectable, absorbable biomaterial composed of a calcium cross-linked alginate solution which undergoes *in situ* gelation after delivery into the infarcted myocardium. Rats (n=60) were subjected to anterior MI and subsequently injected with alginate biomaterial, or collagen, or saline into the infarct. One week later, alginate-treated rats and another group of MI rats were treated with rat fetal cardiomyocyte ($1x10^{6}$ cells) transplantation into the scar. Eight weeks after MI, pressure-volume loop studies showed that LV end-diastolic and systolic volumes were smaller in animals treated by the hybrid therapy, compared with animals treated with injectable collagen or saline. The number of myocardial islands in the scar was similar in animals treated with cell transplantation, with or without injectable alginate. However, relative scar thickness was greater in hearts treated by the hybrid therapy. The portion of fibrosis in the scar was smaller in animals treated with alginate, alginate with cell, or cells only compared with collagen and saline-treated hearts ($p< 0.0001$).
Conclusions: Hybrid therapy of injectable alginate implant with staged transplantation of fetal cardiomyocytes has additive favorable effect on infarct repair and cardiac remodeling after MI.

Hypoxia Inducible Factor-alpha improves the migratory properties of bone-marrow derived mesenchymal cells.

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Background: The efficacy of stem cell therapies for cardiac repair is underpinned by the need to induce appropriate migration and homing to the site of injury. Furthermore, the benefit from self-renewal and differentiation capacities of stem cells is limited unless their migration to target tissues is appropriately orchestrated. Genetic manipulation of stem cells is a feasible approach for this purpose. Hypoxia inducible factor (HIF) plays a pivotal role in controlling angiogenesis, erythropoiesis, vascular tone and cell motility.
Aim: We sought to investigate the effect of HIF1α and HIF2α on the migratory potential of bone-marrow derived mesenchymal cells.
Methods Mesenchymal cells were obtained from Wistar rats and retrovirally modified to express stable forms of eGFP-hHIF1α and eGFP-hHIF2α. Concomitantly, total myocardial protein was extracted from adult rat heart. The migratory capacity of the transduced cells towards cardiac extract (1.7 ug/ml myocardial protein) was tested using TransWell inserts, and compared to that of control mesenchymal cells transduced with eGFP only.
Results: Interestingly, HIF2α transduced cells showed a >2-fold increase in migration capacity whereas eGFP-HIF1α or eGFP only-transduced cells showed no comparable increase.
Conclusions: HIF2α gene confers an enhanced migratory capacity to mesenchymal cells. This crucial functional property may enhance the therapeutic potential of stem cells in cell-based therapies for cardiac repair.

LAMP2 cardiomyopathy: The consequence of impaired autophagy

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Null allele human mutations in the lysosome-associated membrane protein-2 (*LAMP2*) gene encoded on chromosome X produce a range of clinical manifestations affecting the central nervous system, liver and skeletal muscle. In the heart *LAMP2* mutations trigger massive ventricular hypertrophy with unrelenting progression to heart failure and electrophysiological abnormalities including ventricular arrhythmia and conduction defects. In the affected males, LAMP2 cardiomyopathy emerges during childhood and universally causes death by late adolescence or early adulthood. Hypomorphic *LAMP2* mutations, leading to low-level production of a defective protein, cause a predominantly myocardial disease and account for 1-3% of unexplained cardiac hypertrophy in the young. To elucidate the mechanism of LAMP2 cardiomyopathy we introduced one such human mutation (in frame exon 6 deletion) into the mouse *LAMP2* gene. Lysosomes from mutant mice demonstrate abnormal biogenesis, inability to dock with autophagosomes and erroneous trafficking for recycling and degradation. Dusrupted autophagy in the hearts of LAMP2$^{\Delta 6}$ mice triggered marked calcium dysregulation, myocardial necrosis and increased fibrosis, therein accounting for the hypertrophy, arrhythmia and conduction block of human LAMP2 cardiomyopathy.

ZnT-1, a novel regulator of T- type calcium channels mediating a crosstalk between T-type and L-type calcium channels

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Background: ZnT-1 is a transmembrane protein that was studied mainly in the context of zinc metabolism. New findings mark ZnT-1 as an inhibitor of L-type Calcium Channels (LTCC) and as a potent activator of Raf-1 in the Ras-ERK signaling pathway. Recently, we demonstrated that ZnT-1 inhibits the LTCC by direct binding to the LTCC β-subunit. In addition, we found that ZnT-1 expression in the heart is modulated by electrical pacing and ischemia\reperfusion. In the present study we explore the regulatory effects of ZnT-1 on the activity of T-type Calcium Channels (TTCC), which are known to co express with the LTCC in various cells including cardiomyocytes.
Methods and Results: Voltage clamp recordings in *Xenopus* oocytes revealed that ZnT-1 enhances the TTCC current (167.95±9.27 % of control, n=30, $p<0.005$). Biotinylation experiments indicated that ZnT-1 increases the surface expression of the TTCC (457.94±85.8 % of control, n=3, $p<0.005$). Overexpression of inactive Raf-1 abolish the augmentation of the TTCC current by ZnT-1 (103±4.1 % of control, n=25, $p=0.37$). In addition, we found that the ZnT-1 augmentation of the TTCC is inhibited by the expression of LTCC β-subunit. Finely, co-expression of LTCC, TTCC and ZnT-1 led to preferential inhibition of the LTCC with no effect on the TTCC.
Conclusion: ZnT-1 inversely regulates the activity of TTCC and LTCC. ZnT-1 induced augmentation of TTCC activity involves activation ERK-MAPK and increased TTCC surface expression. The interaction of the LTCCβ-subunit with ZnT-1 leads to a crosstalk between LTCC and TTCC. These findings suggest a key role for ZnT-1 as a regulator of cardiomyocytes excitability and calcium homeostasis.

Abbreviated effective refractory period and amplified dispersion of repolarization underlie the development of atrial fibrillation in a canine atrial wedge model of short QT1: mechanism and implications for anti arrhythmic therapy

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Background: Short QT syndrome (SQT) 1, caused by a gain-of-function in IKr, is associated with atrial fibrillation (AF). We examined the cellular basis for the development and treatment of AF in an experimental model.
Methods: Action potentials (AP) were recorded from pectinate muscle (PM) and crista terminalis (CT) regions of coronary-perfused canine right atrial preparations, together with a pseudo-ECG. Spatial dispersion of repolarization (SDR) was determined as the inter-regional differences between the average atrial AP duration measured at 90% and 70% repolarization (APD90 and APD70 respectively). Effective refractory period (ERP) was measured at a pacing cycle length of 500 ms in PM. After achieving maximal ERP and ADP abbreviation with PD-118057 (20 μM), we added either E4031 (5 μM), Lidocaine (20 μM), Quinidine (10 μM) or Isoproterenol (100 nM) to the coronary perfusate. We attempted to induce AF using single extrastimuli under each set of conditions studied.
Results: The IKr agonist PD-118057 (20 μM) significantly abbreviated ERP, APD90 and APD70 in both CT and PM (n = 28). SDR increased from 27± 17 to 51 ± 32 (p= 0.002; n= 28). AF could be induced by premature stimulation only after exposure to PD-118057 26/28 (93%). Lidocaine (n=5) significantly prolonged only ERP and was not effective in preventing induction of atrial arrhythmias. E4031 (n= 5) and Quinidine (n=5) prolonged APD. Quinidine prolonged ERP to a greater extent than E4031 and prevented induction of AF. Isoproterenol further shortened both APD and ERP and facilitated induction of sustained AF in 5/6 preparations.
Conclusions: The IKr agonist recapitulates the electrophysiologic and arrhythmic manifestations of SQT1. Abbreviation of APD, ERP and amplification of SDR all predispose to the development of AF by creating the substrate for development of reentry. Quinidine, but not sotalol or lidocaine, was effective drug in preventing AF in this setting.

Autophagy is required for preconditioning by the adenosine A1 receptor-selective agonist CCPA

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We have previously shown that autophagy plays a protective role in HL-1 cardiomyocytes subjected to simulated ischemia/reperfusion (sI/R). The purpose of this study was to investigate the effect of adenosine receptor agonists on autophagy and cell survival following sI/R using GFP-LC3 infected HL-1 cells or neonatal rat cardiomyocytes.
The A1 adenosine receptor agonist CCPA (100 nM) caused an increase in autophagosomes within 10 min and persisted for 300 min, whereas the A3 agonist Cl-IB MECA had no effect. A significant inhibition of autophagy and loss of protection against simulated ischemia/reperfusion (LDH release) was demonstrated in CCPA-pretreated cells treated with the A1 receptor antagonist DPCPX, the PLC inhibitor U73122, or the Ca(+2) chelator BAPTA-AM. To determine whether autophagy was required for the protective effect of CCPA, cells were transfected with Atg5K130R, a dominant negative inhibitor of autophagy. CCPA prevented LDH release after sI/R, but Atg5K130R abolished this protection. To assess autophagy *in vivo*, mCherry-LC3 transgenic mice were treated with CCPA for 30 min. Fluorescence microscopy of cryosections revealed a large increase in the number of LC3mCherry puncta, indicating the induction of autophagy by CCPA *in vivo*.
Taken together, these results suggest that autophagy contributes to the protective action of adenosine preconditioning.

Rapamycin protects heart cultures against hypoxia via inhibition of SERCA and activation of PKC and MAPK

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Introduction: Rapamycin (sirolimus) is an antibiotic that inhibits protein synthesis through mammalian target of rapamycin (mTOR) signaling and is used as an immunosuppressant. The proposed mechanism for the anti proliferative effect of rapamycin is based on its ability to bind to its intracellular receptor, the FK506 binding protein (FKBP12.6). Ryanodine receptors (RyR2), the major intracellular Ca^{2+} release channel in the cardiac muscle, play an essential role in excitation-contraction coupling by regulating the release of Ca^{2+} from the sarcoplasmic reticulum (SR) for binding to the contractile apparatus. RyR2 channel function is a subject to exquisite levels of modulation via diverse mechanisms, including interaction with accessory proteins such as FKBP12.6. Our goal was to elucidate the protective mechanism induced by rapamycin and to study the interaction between rapamycin and RyR2 in hypoxic rat heart cultures.
Results: Rapamycin inhibited SR Ca^{2+} ATPase (SERCA2A) and $^{45}Ca^{2+}$ uptake into the SR in a dose and time dependent manner in heart cultures that were skinned with saponin and exposed to rapamycin. Cytosolic calcium ($[Ca^{2+}]_i$) was also recorded in cardiomyocytes that were loaded with indo-1 and fluorescent ratio of 410/490 nm was measured. Rapamycin caused a temporary $[Ca^{2+}]_i$ elevation which was accompanied with a decreased of spontaneous contractility. In heart cultures that were exposed to 90 min hypoxia and reoxygenation, rapamycin (10 μM) and ryanodine (2 μM) attenuated by 40-50% LDH (lactate dehydrogenase) or CK (creatine kinase) leakage from cardiomyocytes that were subjected to hypoxia and reoxygenation. Chelerythrine (a PKC inhibitor, 2 μM) - abolished the protective effect of rapamycin, indicating that elevation in cytosolic Ca^{2+} via SERCA2A inhibition is involved in the protective mechanism of rapamycin against hypoxia. Morphological analysis as revealed by desmin immunostaining and MTT measurements confirmed the result of cardioprotection by rapamycin against hypoxia. In order to study the mechanism of cardioprotective effect of rapamycin against hypoxia specific inhibitors of MAP kinases were used: PD 98059 (20μM) an inhibitor of MEK (MAP kinase kinase) and SB203580 (0.5 μM) and SB202190 (20μM) - a selective p38 MAPK inhibitors abolished the cardioprotective effect of rapamycin against hypoxia , as detected by LDH released to the medium.
Conclusion: Rapamycin protects heart cultures against hypoxia via inhibiting SERCA2A that caused cytosolic $[Ca^{2+}]_i$ elevation and induced PKC and MAP kinases activation.

Physiological and molecular evidence of heat acclimation mediated cross-tolerance memory: a lesson from the heart

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Background: (i) Environmental Cross-Tolerance is the protection acquired to a novel stressor via preceding acclimation to a different stressor. (ii) Sporadic data demonstrate that re-induction of acclimation after its loss occurs markedly faster than the initial AC session (iii) Our previous studies substantiated heat acclimation-cardioprotection cross-tolerance; the underling processes are primarily molecular. .
Goal: To test the hypothesis that faster re-induction of heat acclimation cross tolerance (HACT) involves 'molecular memory' linked to epigenetic modifications.
Procedure: Using the *Rattus norvegicus* AC model physiological-integrative, cellular and molecular aspects of deacclimation (DeAC, 30 and 60 d) and subsequent reacclimation (ReAC, 2d) were assessed. Criteria for assessment of DeAC/ReAC were thermoregulatory, and HACT in the heart, comprising infarct size following ischemia/reperfusion insult (I/R) and time to rigor contracture in anoxic cardiomyocytes. To assess epigenetic modifications, histones acetylation (H4, H3) and phosphorylation (H3-Ser10) at the regions of the heat shock response element (HSE) in *hsp*70 and *hsp*90 promoters were measured using Chromatin immunoprecipitation (ChIP). HSE accessibility was analyzed via HSF1-ChIP at the region of the *hsp*70 promoter and measuring *hsp*70 mRNA kinetics following heat stress (HS) using qPCR.
Results: *Ex vivo* assessment of cross-tolerance to I/R or anoxia demonstrated that ReAC only needs 2d vs. the 30d required for the initial development of AC phenotype. A cluster of transcriptionally activated genes among which HSPs, and chromatin remodeler genes, did not resume pre-acclimation levels after DeAC despite the return of the physiological phenotype to its pre-acclimation state, suggesting a dichotomy between the geno- and the pheno- types. ChIP analyses provided evidence of H4 acetylation during AC, DeAC and ReAC and H3Ser10-P in 2d and ReAC groups. The accessibility of the HSE to HSF1 was validated by elevated binding to *hsp*70 promoter and by similar acclimatory mRNA kinetics post HS in the AC, DeAC and ReAC groups.
Conclusion: We argue that chromatin remodeling emerging with AC and retained during DeAC upon ReAC is an upstream regulator of the transcription state, preconditioning to faster cytoprotective acclimatory-memory.

Flow induction of blood-vessel network within engineered tissues

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Introduction: Tissue-engineering field is aimed to produce cell-based substitutes with the use of biomaterials for tissue repair and replacement. To achieve the goal of engineering large complex tissues in the lab, and maintain construct viability following transplantation, vascularization of the construct is essential.
Vascularization is known to be highly regulated by many chemical and physical stimuli, such as flow induced shear-stress. Fluid shear stress is sensed by ECs and plays a crucial role in vascular formation, stabilization, remodeling and function.
In our previous studies we have shown that co-culturing Endothelial cells (ECs) with human embryonic stem cells derived cardiomyocytes on three-dimensional (3D) biodegradable porous scaffolds can provide a way to vascularize the engineered cardiac tissue *in vitro*. Such vascularization can be further promoted by addition of embryonic fibroblasts that differentiate in the presence of the ECs to smooth muscle-like cells (SMCs), and results in self-organization of ECs and SMCs to vessels network within the beating cardiac construct. We have shown that pre-vascularization of the tissue *in vitro* can facilitate its survival and viability *in vivo* upon implantation.
Research objective: In this study, we impose flow-induced shear stress on 3D multicellular scaffolds and investigate the effect of relevant flow profiles on ECs' organization in such scaffolds. The goal of this study is to understand the role of fluid shear stress in endothelial maturation and vascular network formation within 3D tissue-constructs.
Methods: 3D vascular scaffolds were co-seeded with ECs (HUVEC cells) and human fibroblast cells (HFF cells), and were directly perfused with laminar medium flow (average shear stress values of 1 and 3 dyne/cm^2) within bioreactor system. Since it is impossible to measure shear-stress within porous scaffold, 3D computational modeling was deigned for predicting the shear-stress acting on cells; this allowed us to keep them in a suitable range for cellular growth. We examined the influence of fluid shear stress on vessel formation, organization, alignment, length, distribution and stabilization using advanced microscopy imaging, such as Fluorescence and Confocal microscopy, for visualization of the 3D vessels structures.
Results and conclusions: Our preliminary results indicate that scaffolds grown in static conditions and transferred to flow induced shear of 3 dyne/cm^2 generated more lumens which were homogeneously distributed throughout the scaffolds, compared to scaffolds grown in static conditions, which were mainly located in the boundaries of the scaffold. Observation of 3D structure of the vessels using Confocal microscopy, showed elongated, interconnected and well-branched vessel-network. Also, we have shown that under flow conditions vessel network length was much longer in contrast to the length obtained under static conditions.
We hope that this research will enable better control over *in vitro* tissue vascularization processes, which can later be used to engineer larger and viable tissue-constructs for clinical applications.

Plaque neovascularization and its quantification

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Introduction: Atherosclerosis, a disease of the cardiovascular system, is a leading cause of death. Progress of atherosclerosis can be expressed by increase of the intima-media thickness (IMT), and at a later stage, by plaque generation. Recently, studies of the relationship between cardiovascular events and stroke established the presence of the neovascularization inside the plaque as the marker of plaque vulnerability. With the well substantiated concept that inflammation stimulates this neovascularization, the relation between inflammation and vulnerable plaques can be examined. We hypothesize that using contrast US imaging, the neovascularization can be visualized and quantified, as a marker of the proliferation of this vasculature within the plaque. Thus, our research deals with the detection and measurement of the carotid intra-plaque vasculature tree.
Objectives: The goal of this research is to allow the evaluation of the carotid intra-plaque neovascularization. This will help to assess the probability of plaque detachment and to examine the association between the development of the carotid vasa vasorum and the intra-plaque neovascularization.
Methods: Clinical *in vivo* ultrasound images were acquired from 17 patients, several hours before undergoing endarterectomy surgery. 1cc ultrasound contrast agent (Definity) was injected as a bolus, and full ultrasound study was performed. Specimens were collected and transferred to histopathologic examination of vascular and inflammation loads. An algorithm was developed to process these images, which had to cope with poor image quality and low resolution. Image artifacts were removed, as well as translation/rotation of the plaque viewed within each frame. Morphological analysis and tracking methods were implemented. Quantification of the lumen volume of the imaged arterioles enabled the comparison with the final histopathologic evaluation.
Results: Of the 17 histological cases studied, 12 exhibited inflammation. In 75% of these 12 cases, blood vessels could be visualized. About 90% correlation was found between the inflammation severity and the number of the blood vessels. Furthermore, measurement of neovascularization within the plaque is correlated with excessive flow in the vasa vasorum.
Discussion: The relationships between the amount of intra-plaque neovascularization and intra-plaque inflammation and extensive flow inside the vasa vasorum have been examined. The application of ultrasound contrast agent and the utilization of a new algorithm allowed comparison to the histology: high correspondence was found between the neovascularization severity as measured by ex-vivo histology and by in-vivo imaging. Thus, the new tool may help assess the risk of stroke and other cardiovascular events.

A functional role for eotaxin-2 in the initiation and progression of experimental atheroma

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The chemokine eotaxin-2 is a potent chemoattractant for inflammatory cells, the predominant of which are eosinophils. Eotaxin-2 binds to the eosinophil receptor CCL24, also named CCR3, and possesses a potent chemotactic activity for eosinophils. Human and murine atherosclerotic plaques are known to exhibit inflammatory phenotypes where a complex interaction of cytokined and chemokines play a role. We tested the hypothesis that eotaxin-2 plays a causative role in the initiation and progression of atherosclerosis.
Employing reverse-transcriptase PCR analysis, we have shown that eotaxin-2 is abundantly expressed in plaque from apoE knockout (KO) mice. Administration of polyclonal blocking antibodies to eotaxin-2 resulted in a robust reduction of early atherosclerotic plaques in apoE KO mice whereas prolonged treatment of mice with advanced plaques led to atheroma stabilization. A neutralizing monoclonal antibody (1D8) against eotaxin-2, produced in our laboratory, significantly attenuated adhesion of lymphocytes and monocytes as well as heart-derived H5V cells to fibronectin and successfully inhibited their migration towards VEGF. Furthermore, we have shown that 1D8 interferes with binding of eptaxin-2 to the chemokine-recognition site on CCR3. Similar to the polyclonal antibodies, 1D8 significantly reduced atherosclerotic plaques in apoE KO mice, pointing out to the promising therapeutic potential of this monoclonal antibody.
Conclusion Eotaxin-2 represents a novel target in human atheroma and its blockade by neutralizing antibodies is associated with reduced fatty streak accumulation and plaque stabilization in mice**.**

Vessel/myocardium interaction affecting intramyocardial dynamic flow

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Introduction: Myocardial ischemia is the most common cause for morbidity and mortality in the Western world, resulting from intramyocardial hypoperfusion. Investigation of both ischemia underlying mechanisms and of novel protection methods is impeded by the excessive difficulty associated with *in-situ* intramural flow measurements. Computerized flow simulation, an attractive alternative to these measurements, must base on detailed representation of vascular anatomy and physiology as well as reliable description of the temporally- and spatially-varying vessel/myocardium interactions. This goal has not been accomplished before, due to the vast count of coronary segments ($\sim 10^8$), uncharacterized mechanical behavior of *in-situ* vessels and undetermined nature of interaction. The present study attempts to achieve this goal by introducing novel bottom-up network reconstruction, large-deformation stress analysis, and measurements-based interaction mechanisms discrimination.
Objective: To assess, through model simulation, transmural flow and its underlying determinants.
Methods: A precise morphometric representation of the pig left-ventricle microcirculatory network was reconstructed based on statistical morphometric data. Four duplicates of this network were interconnected and positioned at representative intramyocardial layers, hence subjected to different extra-vascular loading. Vessels *in-situ* diameter response to varying loading conditions (myocardial activation, extravascular pressure and axial stretch) was modeled and validated. Segmental flow was modeled by a validated non-linear three-element windkessel. Several interaction mechanisms were investigated according to their fit to measured regional perfusion, segmental intravascular pressure, systolic/diastolic diameter change, and velocity waveforms. Flow under the best fitting mechanism was simulated while separately varying flow driving pressures, vascular compliance and blood viscosity
Results: Only a vessel/myocardium interaction that includes the combined effects of cavity derived interstitial fluid pressure and of contraction dependent intra-myocyte pressure is in good agreement with all experimental data. Such interaction is predicted to compromise subendocardial perfusion to all layers, turn subendocardial and venous intravascular pressures more oscillatory, and affect the longitudinal location of flow phase shift. Stiffer vessels reduce subepicardial perfusion while hardly affecting subendocardial one, effectively elevating endo/epi ratio. Elevated left-ventricular pressure reduces endo/epi ratio by reducing flow mainly to subendocardium.
Discussion: The present study discriminates between several vessel/myocardium interaction mechanisms, and consequently addresses a long-standing controversy. It further allows comprehension of the role of selected mechanisms underlying heterogeneity in perfusion, flow patterns and intravascular pressures. Though not yet fully accountable for pathologies, the introduced computational framework sheds light on basic determinants of coronary flow and on flow sensitivity to clinically approachable indices.

The external-work pressure-time integral relationships and the afterload dependence of frank starling mechanism

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Background: The mechanisms underlying the Frank-Starling Law of the heart are elusive. Despite the prevalent notion that it is afterload independent, isolated fiber studies reveal that the afterload affects the related force length relationship. The study explores the roles of the afterload, in situ.
Methods: The LV was exposed by left-thoracotomy in adult sheep (72.6±8.2Kg, n=8). Pressure transducers were inserted into the LV and aorta. Flowmeter was placed around the aortic root. LV volume was assessed by sonocrystals. Occluders around the aorta and the inferior vena-cava enabled to control the afterlaod and preload. Different afterloads were imposed by partial aortic occlusions. Transient inferior vena-cava occlusions (IVCOs) were preformed at each steady afterload.
Results: A highly linear relationship was found between the external work (EW) and pressure-time integral (PTI) (R^2=0.98±0.01) during each transient IVCO (n=48). These EW-PTI relationships (WPTiRs) were preload independent since the preload had a proportional effect on the EW and PTI at constant afterload. Interestingly, the slope of the WPTiR was afterload dependant. The slope was 33.3±4.1 mJ/(mmHg·s) at baselines and decreased by 1.0±0.50 mJ/(mmHg·s) per 1 mmHg·min/L increase in the peripheral resistance. A unique WPTiR was obtained during both the occlusion and release phases of each IVCO, while two distinct EW-preload or PTI-preload relationships were observed. The same WPTiRs were also obtained for steady state conditions where the afterload was constant and the preload changes were only due to changes in lung ventilation and not an invasive IVCO.
Conclusions: The novel WPTiR ties the Frank (pressure development) and Starling (EW production) phenomena together. The dependence of the WPTiR on the afterload highlights the adaptive control of the Frank-Starling mechanisms to changes in the afterload. Since the WPTiR can be obtained in a minimally invasive manner, it also has the potential to be of clinical use.