MEDICAL UNIVERSITY OF VIENNA

Cardiovascular tissue regeneration and repair

Thesis Program of the Curriculum of
"Doctor of Phylosophy"
N094

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Short description

Regenerative medicine is considered as a key technology of the present and future medicine, with potential new therapies for cardiovascular, skin, neurological, and many other diseases. Majority of the cardiovascular regeneration research focussed on ischemic heart diseases, but it should encompass also other cardiovascular disorders, such as oncologic treatment-induced cardiotoxicity, or congenital and metabolic heart diseases or brain-heart axis repair. In the last twenty years, some evolutionary breakthrough in tissue regeneration could be achieved, including the development of induced pluripotent stem cells (iPS), identification of essential characteristics of regenerative cell types, development of cardiac organoids, tissue engineerimng and 3D printing. Beyond cell-based cardiac repair technology, regenerative medicine involves also the application of cell-free materials such as secretomes of regenerative cells, extracellular vesicles (EVs), growth factors, non-coding RNAs and gene therapies, biomaterials and several types of engineered tissues. Regenerative medicine can be divided into two complementary strategies: 1) stimulation of the endogenous repair mechanisms by applied therapeutics (eg. synthetic or biologically derived factors, genetic and epigenetic modifications) or interventions (eg. ischemic conditioning, cardioprotection), and 2) exogenous regeneration modes through use of cells, tissues, or other biotechnological products, such as advanced therapy medicinal products, ATMPs. In contrast to pre-clinical data, human cardiac regenerative therapies led to a moderate success in improvement of cardiac performance, reduction of infarct size, prevention of left ventricular remodeling and decreased mortality and cardiovascular morbidity. Therefore, it is fundamental to understand the underlying biological mechanisms and translate the therapeutic efficacy gained in pre-clinical experiments to human conditions.

I. Scope

Clinical cardiac regeneration studies. Two decades ago, the use of autologous stem or progenitor cells for tissue regeneration of the human heart led to the expectation of rescue of ischemic damage of the myocytes, enhance vascular density and rebuild injured myocardial tissue. The accumulated data in 2019 indicated, however, that the therapeutic success of these cell-based therapies is modest and the tissue regeneration involves much more complex processes than cell-related biologics. Accordingly, several new cell types, paracrine factors and other new biologics, such as exosomes or non-coding RNAs are used in pilot clinical trials, mostly selecting patients with ischemic heart failure, aiming to facilitate the reprogramming of the hosting cardiac cells at the site.

Small animal models of cardiac regeneration. Small animal models of cardiac regeneration are necessary to investigate basic mechanisms of cardiac repair. The advantages of these disease models are the relatively low cost, allowing larger sample sizes and modeling of disease over an entire life cycle.

Preclinical and translational models of cardiac repair. The clinical cardiac cell therapy trials showed mixed clinical success as compared with the promising small animal experiments. The disappointing results led to the recognition, that better translational experiments are necessary to simulate the ischemic heart diseases and effect of cardiac regeneration therapies. Large animal models are mandatory to simulate of human diseases and therapies, since the cardiovascular anatomy, physiology and pathophysiology of the large animals are more similar to humans.

Cardiac regeneration in children and congenital heart diseases. Pediatric cardiac tissue samples have higher number of residual cardiac progenitor cells, with enhanced cardiac differentiation and proliferation capacity in the neonatal age compared to adult hearts, suggesting higher regenerative potential of the pediatric heart. According to the difficulties of pediatric heart transplantation, and the high mortality of severe heart failure of any origin in children, an intensive search after new regenerative therapies in children is on-going.

Pre-clinical, molecular and clinical imaging of cardiovascular regeneration. Molecular imaging aims at non-invasive visualization of cardiac regenerative processes at the molecular level. This includes visualizing the diseased tissues as well as tracking cell therapeutics and following processes of regeneration. Imaging of vasculature can further give insight into the regenerative processes and underlying pathologies.

Novel biologics I: non-coding RNAs in cardiac repair. In the cardiovascular system mRNAs, small noncoding RNAs (microRNAs, miRNAs), long noncoding RNAs (lncRNAs), proteome and exosomes from outside the cell interact with signaling pathways and network of pathways in pathophysiological condition, such as acute myocardial infarction or chronic heart failure with left ventricular remodeling.

Novel biologics II: Extracellular vesicles. Cell-to-cell communication plays an essential role in the maintenance of cardiac homeostasis. Recent research has highlighted the role of extracellular vesicle (EVs) in the heart and that it may exceed the importance of other paracrine signaling such as cytokines. Exosomes are a class of EVs that are generated by inward budding in multivesicular bodies (MVBs). On the other hand, the second class of EVs comprise ectosomes, which are produced by direct budding from the cell membrane. Both classes of vesicles consist of a lipid bilayer and a specific set of proteins, and nucleic acids

(miRNA, other small RNAs, mRNA and even double stranded DNA). Their specific contents and the quantity are regulated by the cellular environment.

Novel biologics III: secretomes and other paracrine factors. Stem cells secret several types of molecules, like growth factors, chemokines, cytokines or exosomes. The secretome of cardiac implanted cells induces several effector mechanisms of cardiac regeneration, which is necessary for tissue repair and remodeling. The paracrine history led to the idea of cell-free therapy, with the undoubtable benefit of freedom from complicated cell manipulation to retain the survival status of the autologous or allogeneic stem cells, transplanted in humans.

Regeneration of vascular tissues. There are two important aspects of vascular regeneration, namely self-regeneration on the one hand and efforts in tissue engineering that apply knowledge gained from our understanding of self-regeneration in order to design fully functional vascular prostheses for in vivo use. In order to achieve this aim, the complex network of different cells co-operating in the regenerative response to tissue injury and vascular damage has to be understood. Vascular tissue engineering aims to model in vivo blood vessels in order to design fully functional vascular network. Major issues in such efforts are to successfully prevent thrombogenicity, to achieve favorable biomechanical properties and to exploit beneficial and limit detrimental effects of inflammation.

Molecular mechanisms of cardiovascular regeneration. Besides investigation of protein-coding genes (<3% of the human genome), current research increasingly focuses on non-coding RNAs (ncRNA) (comprising up to 98% of the human genome), which are powerful regulators of several homeostatic processes, tissue and cellular functions, and repair, such as post-transcriptional gene and epigenetic control and nuclear genome organization in the cardiovascular system.

Network Approaches in Medicine. Networks Medicine provides a systematic mapping of omics data, based on high-throughput analyses of unbiased transcriptom, proteome, etc. data, creating an 'interactome' maps, aiming at the investigation of disease-associated genetic and epigenetic perturbations on the network levels. Analysis of "omics" (genomic, transcriptomics, but also proteomics, image-omics, etc) data is associated with personalized medicine, although the role of "omics" on the outcomes of cell-based cardiac regenerative therapy is not sufficiently understood.

3D Printing of cardiovascular tissues and its role in regeneration. 3-dimensional bioprinting aims at creation of clinically usable 3D construct, that is immediately implantable into the human body. The ideal 3D scaffold for cardiac tissue engineering is biocompatible, and simulates the physiologic characters of the targeted organ. Several types of 3D bioprinted scaffolds have been constructed and applied under experimental conditions, such as occluder devices, vascular grafts, coronary arteries or valve prostheses. By using 3D imaging technologies of patients (CT or MRI), a personalized 3D scaffold printing became possible.

Cardiovascular Regeneration with decellularized grafts and valve replacement. Decellularization of heart valves was introduced in Europe over a decade ago. This process removes all donor cells and DNA, which hinders immune response of the recipient and enables host cells to repopulate the extracellular matrix. The Medical University of Vienna started a homograft harvesting facility and implanted the first decellularized graft in 2015. Since then, a steadily increasing number of decellularized heart valves are implanted.

Cardio-Oncology

Anticancer therapy leads to multiple types of myocardial damages, resulting in cardiac fibrosis, arrhythmias, fulminant immune myocarditis, pericarditis, and thrombotic/ischemic events. Cardiotoxicity research focusses on the evaluation of basic mechanisms of myocardial injury caused by cytotoxic or immunotherapy drugs, and searching of biologics for prevention, and repair the pharmacological damaged heart.

Cardio- and Neuroregeneration

Both vitally important organs, the brain and heart have a limited ability to self-regeneration after tissue or vascular injury. Even if several molecular processes of the organ damage (such as redox paracrine signaling) are similar, less is known about the brain-heart interaction and the multiple coupling between cardiovascular and cerebrovascular diseases, and common regenerative processes.

II. Courses

1.	**Propedeutics**	**6 semester hours**
2.	**Basic Lectures**	**4 semester hours**
3.	**Thesis Seminars**	**8 semester hours**
4.	**Journal clubs and progress reports**	**12 semester hours**

1. Propedeutics

General for all N094 students

2. Basic lectures (2 semester hours/semester, 3. and 4. Semesters):

Basic Course 1: Clinical cardiac regeneration studies
Basic Course 2: Small animal models of cardiac regeneration
Basic Course 3: Preclinical and translational models of cardiac repair
Basic Course 4: Cardiac regeneration in children and congenital heart diseases
Basic Course 5: Molecular and clinical imaging of cardiovascular regeneration
Basic Course 6: Novel biologics I: non-coding RNAs in cardiac repair
Basic Course 7: Novel biologics II: Extracellular vesicles: exosomes and microvesicles
Basic Course 8: Novel biologics III: secretomes and other paracrine factors
Basic Course 9: Regeneration of vascular tissues
Basic Course 10: Molecular mechanisms of cardiovascular regeneration
Basic Course 11: Network Approaches in Medicine
Basic Course 12: 3D Printing of cardiovascular tissues and its role in regeneration
Basic Course 13: Cardiovascular Regeneration with decellularized grafts and valve replacement
Basic Course 14: Cardio-Oncology
Basic Course 15: Cardio- and Neuroregeneration

3. Thesis seminars

In thesis seminars, the relevant on-going work of the PhD students will be discussed and critically evaluated (2 hours per week per semester). The aim of the thesis seminar is the interactive work among PhD students and supervisors and exchange the knowledge and methods.

4. Journal Clubs/Progress reports

The Journal Clubs (paper critical review) will be held weekly by the principal investigators of the program. They will discuss the actual relevant literature, comparing to the past published data.

III. Participating Principal Investigators / Supervisors

Name	Affiliation	Field	Role
Andreas, Martin	Division of Cardiac Surgery, Department of Surgery, MUW	Decellularized scaffolds for cardiac repair	Lecturer
Goliasch, Georg*	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, valve replacement therapy	Principal Investigator / Senior Supervisor
Holnthoner, Wolfgang	Endothelial Cell Group, LBI for Experimental and Clinical Traumatology, AUVA Research Centre, LBI	Novel biologics I: exosomes and microvesicles	Lecturer
Hülsmann, Martin *	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, heart failure	Principal Investigator / Junior Supervisor
Kiss, Attila *	Center for Biomedical Research, MUW	Small animal models of cardioprotection and repair	Principal Investigator / Senior Supervisor
Mascherbauer, Julia *	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, myocardial and valve diseases,	Principal Investigator / Senior Supervisor
Menche, Jörg *	CeMM Research Center for Molecular Medicine, Vienna (CeMM)	Network Medicine	Principal Investigator / Senior Supervisor
Michel-Behnke, Ina	University Hospital for Children and Adolescent Medicine, Division of Pediatric Cardiology, Pediatric Heart Center, MUW	Cardiac regeneration in pediatric patients	Lecturer
Moscato, Francesco *	Center for Medical Physics and Biomedical Engineering	3D printing in cardiovascular medicine	Principal Investigator / Senior Supervisor
Pavo, Noemi *	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, translational animal models	Principal Investigator / Senior Supervisor (cand.)
Pavone-Gyöngyösi, Mariann *	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, translational animal models	Principal Investigator / Senior Supervisor
Podesser, Bruno *	Center for Biomedical Research, MUW	Small animal models of cardioprotection and repair	Principal Investigator / Senior Supervisor
Radtke, Christine *	Department of Plastic and Reconstructive Surgery, MUW	Neuro-cardio injury and repair axis	Principal Investigator / Senior Supervisor
Scherer, Thomas *	3rd Dept. Internal medicine, MUW	Metabolic disorders and cardiovascular systems	Principal Investigator / Senior Supervisor
Schernthaner, Gerit	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, translational animal models	Lecturer
Schmid, Johannes *	Institute of Vascular Biology and Thrombosis Research, MUW	Vacular inflammation and thrombosis	Principal Investigator / Senior Supervisor

Slezak, Paul	Pre-clinical imaging Group, LBI for Experimental and Clinical Traumatology, AUVA Research Centre, LBI	Pre-clinical in vivo and ex vivo imaging	Lecturer
Weitzer, Georg *	Department of Medical Biochemistry, Max F. Perutz Laboratories, MUW	Molecular mechanisms of cardiovascular regeneration	Principal Investigator / Senior Supervisor
Winkler, Bernhard Michael	Krankenhaus Nord, Vienna	Cardiac surgery, translational animal model, tissue engineering	Lecturer
Wojta, Johann *	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Vascular tissue regeneration	Principal Investigator / Senior Supervisor
Wolbank, Susanne	Human Stem Cells Group, LBI for Experimental and Clinical Traumatology, AUVA Research Centre, LBI	Molecular imaging, paracrine factors	Lecturer
Zellniker, Thomas	2nd Dept. Internal Medicine, Division of Cardiology, MUW	Clinical cardiology, risk factors	Lecturer

* competitive grants

IV. Experimental techniques

Device name	**Description**	**Department/Center**
Sunrise Multiplate reader, ELISA	ELISA assay	Internal Med. II. Cardiology
Olympus IX83 Microscope	Histology assesssment	Internal Med. II. Cardiology
Cell culture	Cell culture	Internal Med. II. Cardiology
GE portable echocardiographic equipment	Echocardiography for large animals	Internal Med. II. Cardiology
Medrad Vistron CT WHU600	contrast agent injector	Internal Med. II. Cardiology
Siemens Magnetom Vision Plus 1.5T	MRI equipment	Internal Med. II. Cardiology, in cooperation
QIACube	RNA, DNA isolation	Internal Med. II. Cardiology
Myograph MDT	Assessment of vascular function in aorta and in coronary arteries in rodents	Center for Biomedical Research
Hugo-Sachs –Isolated working heart system, AD instrument – Invasive hemodynamic assessment	Assessment of cardiac function in vivo and ex vivo	Center for Biomedical Research
PET/MRI/SPECT	Assessment of cardiac function in vivo (perfusion, viability, inflammation etc).	Center for Biomedical Research share with Dept of Nucl Medicine
Cell culture	Cell culture	LBI Trauma
Zeiss Fluorescence microscope	Cell culture imaging	LBI Trauma
Zeiss Confocal microscope	Cell culture imaging	LBI Trauma
Scanco50 µCT	High resolution ex vivo	LBI Trauma
Maestro in vivo fluorescence imaging	Non invasive	LBI Trauma
Xenogen in vivo luminescence imagine	Non invasive	LBI Trauma
Laser Doppler Imaging scanner	Non invasive Superficial perfusion	LBI Trauma
Stereoscopic 3D camera	3D wound assessment	LBI Trauma

In vivo µCT Scanco	Non invasive rat, mouse	LBI Trauma
Ultracentrifuge	Microparticle enrichment	LBI Trauma
Nanoparticle Tracking Analysis (NTA, Particlemetrix) Flow Cytometer (Cytoflex; Beckman Coulter)	Microparticle characterization Flow Cytometer	LBI Trauma
Homograft Harvesting Facility	decellularized tissue available for recellularization	Department of Cardiac Surgery
Ultra-High-Speed Sorting & High-End Analysis of biological materialFlow Cytometer and Sorter	Flow Cytometer	Core Facilities of the MUW
Affymetrix 7G GeneChip System Affymetrix GeneTitan Illumina HiSeq2000 System Roche GSJunior 454 Fluidigm Applied Biosystems Fast Real-Time System Illumina MiSeq	Microarray Gene Expression Analysis High Throughput Genotyping Copy Number-Variationsanalysis	Core Facilities Genomics of the MUW
Rapiflex MALDI mass spectrometer.	Protein Analysis Sevices	Proteomics Core Facility of the MUW
Laser Scan-, Fluoreszence- & Light-Microscopy	High resolution imaging of tissue sections or cells, 3D and 4D acquisition, visualization of protein-protein interactions (like FRET) and live cell imaging.	Imaging Core Facility of the MUW