



DOCTORAL PROGRAM IN BIOENGINEERING

Chair:

Prof. Andrea Aliverti

The main objective of the PhD Programme in Bioengineering is to prepare the PhD candidates to the development of high level engineering problem-solving abilities in biomedical, healthcare and life sciences, inside research groups or in private/public industrial contexts, through a strong interdisciplinary training bridging engineering and medical/biological knowledge.

During the PhD, the candidates develop a scientific research project dealing with a complex problem (which can be at different scales, from the molecular and the cellular levels to living organisms up to biomedical systems) and investigate original methods, devices, and systems with different purposes: increasing knowledge, proposing innovative methods for diagnosis and therapy as well as improving healthcare and daily life structures and services. At the end of the PhD programme, the candidate are expected to be able to carry out innovative projects in the Bioengineering field, by proposing new methodological and technical solutions and properly evaluating the technology impact in healthcare, life science and biomedical industry.

Research is performed through theoretical and experimental activities in four major areas: biomimetic engineering and micro-nano technologies; rehabilitation engineering and technology; technologies for therapy; physiological modelling and non-invasive diagnostics.

More specific areas include but are not limited to: molecular and cellular engineering, biomaterials, tissue engineering, bio-artificial interfaces and devices, neuro-prostheses, movement analysis, cardiovascular and respiratory system bioengineering, central nervous system signal and image processing for rehabilitation, biomechanics, computational fluid-dynamics, computer assisted surgery and radiotherapy, artificial organs, implantable devices, biomedical signal and image processing, E-Health, bioinformatics, functional genomics and molecular medicine.

Since 2013, the PhD Program in Bioengineering is organized with an inter-departmental structure. Faculty members of the PhD Advisory Board belong to two Departments of the Politecnico di Milano: DEIB (Department of Electronics, Information and Bioengineering) and CMIC (Department of Chemistry, Materials and Chemical Engineering "G. Natta").

PhD candidates (who are, in average, 15-20 per year) develop their

PhD research programs within experimental laboratories located at the Politecnico di Milano or outside it, typically biomedical research centers and hospitals.

When the research is performed within the Politecnico, PhD candidates are usually assigned to one of the following laboratories belonging to the Department of Electronics, Information and Bioengineering (DEIB) and Chemistry Materials and Chemical Engineering Department (CMIC): Laboratory of Biological Structure Mechanics (LaBS, CMIC), Laboratory of movement analysis "Luigi Divieti" (DEIB), Medical Informatics laboratory (DEIB), Neuroengineering and medical robotics Laboratory (NearLab, DEIB), Biosignals, Bioimaging and Bioinformatics Lab (B3 lab, DEIB), Biomaterials laboratory (CMIC), Biomedical Technology Lab (TBMLab, DEIB), Experimental Micro and Biofluid dynamics (μ BS Lab, DEIB), Computational Biomechanics Lab (DEIB), Biocompatibility and Cell culture Lab (BioCell, CMIC), Bioreactors Laboratory (CMIC). The Istituto di Elettronica, Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT) of the Consiglio Nazionale delle Ricerche (CNR), which is located at DEIB, represents another possible option.

Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the PhD candidate training. The candidates are encouraged to carry out part of their research activities in contact with other research groups, preferably abroad through periods of at least three months spent in laboratories where the candidate can acquire further skills to develop his/her research work and thesis.

Collaborations that may involve the PhD students are presently active with several national and international research and academic Institutions. Very often, the involvement of industrial and clinical partners facilitates the technological transfer of applied research into industry and clinical applications.

The educational offer includes *ad hoc* advanced courses specifically designed for the PhD in Bioengineering. The offer includes also the school of the National Bioengineering Group, which is held yearly for one week in Bressanone (Bz). Every year, the School is focused on different topics. As examples, the themes of the last years have been: Neuroscience, robotics and intelligent machines (2006), Computational Genomics and Proteomics (2007), Wearable Intelligent Devices for Human Health (2008), Bioengineering for Cognitive Neurosciences (2009), Synthetic biology (2010), Neuroinformatics (2011), Biomedical devices from research to market (2012), Regenerative medicine (2013), From functional recovery to artificial organs (2014).

The PhD Board of professors ('PhD Board') is composed by highly qualified and active researchers in Bioengineering, belonging to DEIB and CMIC. The PhD Board is responsible of all the candidate's activities. The competencies of Faculty members cover a wide spectrum of research fields. This allows a continuous updating of the PhD program and ensures that the PhD candidates are involved in innovative work.

COMPOSITION OF THE PHD BOARD

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The PhD Programme in Bioengineering relies also on an Advisory Board Member, formed by distinguished experts coming from R&D industries, research and clinical centers, in order to ensure that the goals of the PhD Program are in line also with the needs of non-academic world.

Presently, the members of the Advisory Board are Emanuele Gatti (CEO of Fresenius Medical Care, Homburg, Germany), Ferdinando Grandori (Consiglio Nazionale delle Ricerche, IEIT, Italy), Antonio Malgaroli (Head of Molecular and Cellular Physiology Lab, IRCCS San Raffaele, Milano, Italy), Ivan Martin (Head of the Tissue Engineering Lab, at the University Hospital Basel, Switzerland)

PATIENT-SPECIFIC MODELING OF THE CARDIOVASCULAR SYSTEM FOR SURGICAL PLANNING OF SINGLE-VENTRICLE DEFECTS

Alessia Baretta - Relatore: **Prof. Giancarlo Pennati**

Congenital heart diseases are cardiac malformations consisting of only one effective or functional cardiac pumping chamber (the single ventricle, SV).

SV defects, such as hypoplastic left heart syndrome and tricuspid atresia, require a three-staged surgical approach, called the Fontan procedure, to separate the systemic and pulmonary circulations.

Since the early days of the Fontan procedure, in vitro, in vivo, analytical and computational techniques (including computational fluid-dynamics [CFD] models) have been developed to investigate the complex hemodynamics of the Fontan circulation.

In the present work, CFD techniques are used for the planning of staged surgical treatment of SV malformations. The present work is part of the international Transatlantic Networks of Excellence in Cardiovascular Research Program funded by the Fondation Leducq (Paris), entitled Multi-scale modeling of single ventricle hearts for clinical decision support.

First, pre-operative OD models for Stage 2 surgical planning are built.

Two kinds of models are considered: a closed-loop pure OD network of the whole cardiocirculatory system, based

on the patient-specific main hemodynamic features, and an open-loop multi-domain (3D-OD) model of the pulmonary system, describing in detail the region of Stage 2 surgery.

For each patient, clinical data consisted in catheterization-derived pressure tracings, MR (magnetic resonance) flow tracings and echocardiographic Doppler velocity tracings.

The closed-loop OD model of Stage 1 circulation comprises 4 main peripheral blocks describing the upper/lower body and right/left lung circulation, adopting a 'typical' heart model for Stage1 patients, tuned manually basing on literature works to fit all patients under study. The aim of such patient-specific OD modeling is to prescribe proper boundary conditions to the 3D post-operative geometries, integrated in a multi-domain model, where different surgical options will be compared.

The pre-operative open-loop multi-domain model of the lungs, instead, is built to calculate the impedance downstream all pulmonary branches included in the 3D model, to be integrated in the post-operative models used for surgical planning.

A multi-step approach was implemented to estimate the parameter values of RCRCR blocks downstream the outlet

branches of the 3D model, to be integrated in the 3D-OD model developed in Chapter 3. All simulations were performed by the partners of the Transatlantic Project at INRIA.

OD models built for the whole circulation, described above, were coupled to two different 3D models of the surgical site. The pre-operative anatomical reconstruction was manipulated in order to generate virtual post-operative scenarios. Indeed, two surgical options were virtually performed for each patient: bi-directional Glenn (bG) and hemi-Fontan (hF). In the case of the bG geometry, the SVC-RPA anastomosis was recreated by virtually resecting the SVC from the atrium and adjoining it with minimal movement to the RPA. For the hF geometry, a portion of the atrium was removed from the 3D volume so to create the 'bulging patch' typical of this surgical configuration, the size of which was determined in agreement with the surgical team who performed the operation.

This procedure was performed by the research partners at Great Ormond Street Hospital, London, UK. Each outlet of the 3D model was connected to a RCRCR pulmonary block obtained through the open-loop model described previously; the SVC line of the OD model was disconnected from the atrium

and connected to the SVC inlet section of the 3D model; the shunt block was removed.

First, patient models were tested at rest conditions (using HR, SVR and PVR recorded at the time of the clinical measurements), then at conditions with increased blood flow and/or heart rate ('active state' and removal of the possible stenosis).

In Chapter 4, two specific clinical cases of post-Stage 2 patients are presented. In such configurations, Stage 2 circulatory network serves as pre-operative condition to two different kinds of treatments:

- in the first clinical case, a Stage 3 surgical planning is performed, following a workflow analogous to that presented in Chapter 3 for Stage 2 surgical planning, and three different TCPC geometries are compared. Moreover, respiration effects on the hemodynamics in the different postoperative options are studied. The effect of the respiration is tested in presence of exercise conditions, simulated by increasing the heart rate, and by reducing pulmonary and lower limb vascular resistances.
- the second clinical case consists in a patient diagnosed with veno-venous collateral vessels 4 month after Stage 2 surgery. In this study, i) the patient's cardiovascular network at 4 months after Stage 2 surgery was modeled thanks to the acquisition of clinical measurements post-operatively and taking into account the body growth, and ii) the closure of collateral vessels was simulated. This study shows the differences between pre and post-operative acquired data that may be explained

by adaptation phenomena occurred after Stage 2.

In the final chapter, a preliminary development of an identification process based on clinical available data is developed.

Simplified sub-models are identified thanks to the available clinical tracings that allow to decouple the sub-model from the rest of the circulation.

Then, the integration of the sub-model in a more complex model of the whole circulation is accomplished only subsequently, since a direct identification of all model parameters would lead to multiple possible solutions.

Since the heart parameter tuning is the crucial part, this Chapter is focused on the study of suitable methods to identify heart properties to embed in the circulatory model. More precisely, parameters identified for a submodel of the single ventricle or of the whole heart may be used as step preliminary to the identification of the closed-loop circulatory network. The study is divided into two main parts: first, the feasibility of parameter identification based on PVL data is presented; secondly, a sub-model of the heart is built and parameters are identified through a two-step method and the obtained parameters are integrated in a model of the full circulation thus limiting the range to span.

The model of the sole ventricle is composed of six parameters, while full time-varying tracings of ventricular volume and pressure are used, the former as input of the model, the latter as target quantity.

However, the high uncertainty on clinical PVL tracings is a big issue in handling such data. For this reason, a method based

on more reliable clinical data is preferable.

The heart sub-model parameters are identified through a preliminary implementation of a robust optimization approach based on clinical data of pressures, flows and volumes measured on the patients and relative uncertainty.

Clinical data available, consisting in flow time tracings and mean values, cycle-averaged pressures and end-diastolic volumes, were used in different ways in the model, in particular as i) boundary conditions prescribed in the open-loop model, ii) target quantities to match as the goal of the optimization, iii) constraints on certain parameters values in order to assure that they are physically meaningful, and iv) prior knowledge on measurements available on certain variables. Afterwards, the obtained heart parameters are integrated in a model of the full circulation, giving a 'prior knowledge' to the heart behavior.

A two-step approach was chosen to find the parameter set that best matches clinical data. First, Adaptive Markov Chain Monte Carlo (MCMC) was employed to obtain the distributions of the model parameters. Then, Nelder-Mead hill-climb optimization is performed from the parameter set that was found to maximize the posterior distribution during the previous MCMC iterations. The identification process was performed by means of a MATLAB code written in collaboration with experts at UCSD (University of California San Diego).

A MULTISCALE AND TRANSLATIONAL APPROACH FOR THE CHARACTERIZATION OF LONG QT SYNDROME TYPE 1, 2 AND 3

Vlasta Bari – Supervisors: Prof. Sergio Cerutti, Prof. Alberto Porta

Background and aim

Long QT syndrome (LQTS) is an inherited disease whose main clinical manifestation is a prolonged QT interval on ECG. The reasons for the relevant interest in this disease are its dramatic clinical manifestations, as syncope, ventricular fibrillation and sudden death, but also the existence of symptomatic subjects and siblings almost never developing symptoms. Among the 13 different mutations leading to LQTS identified so far, all affecting cardiac ion channels and all threaten with beta-blockers (BB), this work will focus on the main three variants of the pathology. LQT1 (45% of all LQTS patients) is due to a mutation on the slow part of delayed rectifier potassium current channel, with symptoms precipitating in case of increased sympathetic activity as during physical exercise, mainly during daytime. LQT2 (35% of LQTS patients) is due to a mutation on the rapid part of delayed potassium current, with arrhythmias triggered in case of sympathetic overactivation due to sudden emotional stress or auditory stimuli. LQT3 characterizes only the 10% of patients but is the more lethal. Due to a mutation on the gene encoding the sodium current, in this case events occur during vagal hyperactivation, mostly

during rest and sleep.

It is well known that autonomic nervous system (ANS) plays an important role in triggering fatal arrhythmias in LQTS, but its role as a risk modifier in LQTS is less clear.

The aim of this work was to exploit different tools in time, frequency and information domain in order to characterize the autonomic control of LQTS subjects and improve risk stratification accounting for genotype and phenotype. Findings obtained in LQT1, LQT2 and LQT3 humans were compared with the aim of providing a complete framework about autonomic control in LQTS. A translational process was performed thanks to telemetric ECG recordings in a Δ KPQ-LQT3 transgenic murine population, that were compared with LQT3 results in men.

Methods of analysis

Time domain indices, such as mean and variance of HP and QT and corrected QT evaluated according to the Bazett's correction, were calculated. An autoregressive model was exploited to perform spectral analysis. The model order was optimized according to the Akaike information criterion and the power spectrum was factorized in frequency components according to the residual method. A component

was labeled as low (LF) or high frequency (HF) if its central frequency was respectively included in the band 0.04-0.15 Hz or 0.15-0.5 Hz. Spectral analysis was performed also on mice HP variability series, with HF in the range 1-5 Hz. The power of mice and men HP variability in HF band was taken as an index of vagal modulation directed to the sinus node while the power of QT variability in LF band was taken as an index of sympathetic modulation directed to the ventricles.

To assess the overall complexity of sympathetic and vagal control, a refined multiscale entropy (RMSE) analysis was performed on HP and QT variability series, consisting in three steps: i) elimination of the fast temporal scale of the series through a low-pass Butterworth filter; ii) undersampling the series with a factor τ thus reducing its length from N to N/τ at each scale factor τ (for $\tau=1$ the time series is the original one); iii) assessing complexity at each τ through Sample Entropy, calculated with a tolerance r equal to 0.15 times the standard deviation of the series, with embedding dimension $L=3$ and time shift between samples equal to 1.

RMSE was calculated with τ from 1 to 12, introducing a scale pooling that divides the scale factors in three classes

and then averaging the values over the three classes: $\tau=1$, accounting for all the time scales characterizing the series; $\tau=2-4$ corresponding to medium time scales, progressively filtering HF band activity; $\tau=5-12$ corresponding to long time scales, mainly accounting for activity in LF band.

Empirical mode decomposition (EMD) allows to decompose a time series in its oscillatory modes, the so-called intrinsic mode functions (IMFs). In order to improve short time scale results, in this dissertation a new EMD-based filtering approach was proposed, constituting in computing only the first IMF and subtracting it from the original HP and QT series, thus obtaining a low-pass filtered version of the series. Sample Entropy was then computed over the low-pass EMD filtered series and results were compared with those derived from RMSE.

Protocols and data analysis

The database was composed by more than 100 24h Holter recordings from: 34 LQT1 subjects, divided into asymptomatic (ASYMP) and symptomatic (SYMP) together with 14 non mutation carriers (NMC) from the same family line, 16 LQT2 subjects divided into ASYMP and SYMP and 12 ASYMP LQT3 subjects. Some of the recordings were acquired in absence and some others in presence of BB therapy. Analyses were performed during daytime and nighttime. Several experimental protocols were implemented, accounting for genotype and phenotype, ANS-related circadian variations and effect of BB therapy. Ten Δ KPQ-LQT3 mice were

instrumented and acquired in telemetry together with 10 wild type (WT) littermates. Two-way protocols were implemented to evaluate circadian variations and the effect of pharmacological challenges performed with propranolol, atropine and propranolol and atropine together in LQT3 and WT animals. A translational process was completed comparing circadian variations and the effect of BB therapy in LQT3 men and mice.

Appropriate statistical analysis was carried out in agreement to the protocols.

HP was approximated as the temporal distance between two consecutive R peaks on the ECG. QT was approximated as the temporal distance between the R peak and the T-wave end. Missed or ectopic beats were corrected through cubic spline interpolation. Sequences of 5000 consecutive beats were chosen for each period of analysis except during mice pharmacological challenges where 3000 beats epochs were extracted. Time and frequency domain analyses were carried out iterating the analyses over 250 beats with 50% overlap and taking the median of the distribution of each parameter as representative for the whole series. Complexity analyses were computed over the entire series.

Results

Results showed that ASYMP LQT1 patients had a blunted vagal control and active sympathetic regulation, that makes QT adaptable to sudden HP changes. This feature is thus protective. ASYMP LQT1 had also a lower complexity of cardiac control at medium

and long time scale, suggesting another protective factor. ASYMP LQT2 individuals were characterized by a higher vagal modulation, able to limit sympathetic overactivation that could lead to arrhythmias. Complexity analysis revealed that ASYMP LQT2 patients exhibited a higher complexity of cardiac control at medium and long time scale, leading to an opposite conclusion with respect to LQT1 about the protective role of complexity in the LQTS variant. ASYMP LQT3 patients were characterized by a high vagal modulation, confirming the increased risk for events during night in this group. LQT3 mice was found to be a good translational representation of the mutation in men since mice and men showed similar results in terms of HP increase during sleep/rest periods and in terms of long time scale complexity reduction under BB. Finally, BB appeared to be protective in all variants in different ways.

Conclusions

Spectral analysis typified the ANS state, RMSE quantified the complexity of cardiac regulation as a function of the temporal scales and the EMD-based filtering procedure reduced computational costs of complexity analysis compared to RMSE.

Although the main clinical manifestation of the pathology is similar in all the considered variants, the proposed tools suggested that patients affected by LQT1, LQT2 and LQT3 are characterized by different ANS profiles and some ANS profiles are more favorable than others to reduce the risk of life threatening events.

ADVANCED HUMAN-ROBOT COOPERATION IN NEUROSURGERY

Elisa Beretta – Relatore: Elena De Momi - Tutor: Pietro Cerveri

Over the last decades, neurosurgery has greatly benefitted from the introduction of image-guided techniques and robotic devices. Thanks to their superior resolution, geometric accuracy and indefatigability, robotic systems are mainly used as an accurate and repeatable alignment tool during keyhole neurosurgery. Conversely, open-skull procedures for brain resection/disconnection are traditionally performed free-hand with intraoperative physiological monitoring techniques to identify the functional (eloquent) cortical/subcortical areas, which has to be preserved during the surgery. In particular, direct electrical brain cortex stimulation encompasses the repetitive execution of target reaching gestures on delicate tissue (Figure 1). The conventional approach can benefit from the introduction of a cooperatively controlled robotic assistant, to provide increased positional accuracy and reduce the surgeon's fatigue during the holding phase, when the tool is in contact with the brain tissue. Moreover, it could allow the acquisition of the target positions and guide the surgeon towards the recorded sites, thus increasing the reliability of the intraoperative monitoring technique. In this thesis, we investigated

and developed new methodologies for human-robot and robot-tissue interaction control, specifically designed to augment surgeon's skills during cooperatively assisted targeting tasks on soft tissues. Differently from the standard force-to motion control schema, the control approach proposed exploited the high compliance of a redundant flexible joints industrial manipulator. The validation was performed in a realistic setup with brain-mimicking phantoms (Figure 2), enrolling naïve users as well as novice and expert neurosurgeons. The research was focused on these particular research topics:

(i) *investigate the best control*



1. Intraoperative brain mapping of the motor cortex during glioma surgery. The neurosurgeon is performing the stimulation (up) while the electric brain activity (down right) is recorded from superficial electrodes placed on the cortex (down left) in order to detect the occurrence of unwanted stimulation-induced seizures.

strategy for a comfortable and effective cooperation during patient targeting approaching. Transparency quantifies the ability of a robot to follow human movements without any human-perceptible resistive forces. On the contrary, the ability to approach a target with high accuracy depends on the robot's ability to apply resistance against environmental disturbances. In order to respect the clinical accuracy requirements, while allowing a comfortable cooperation, the surgical robotic assistant should be able to automatically adapt its dynamics during the guidance in the operating theatre. A novel variable damping controller is designed to enhance the performance of the surgical hands-on robotic assistant in terms of ease of use, intuitive guidance and effectiveness during targeting tasks. The experimental evaluation of this and two well-known impedance controllers with fixed dynamic parameters was carried out during predefined reaching tasks towards registered targets on a calibration board. The reaching task was shown under laboratory condition to result in reduced targeting error, which guarantees the respect of the position accuracy requirement (1mm), and user efforts, which ensure that assisted tool trajectories feel natural to the user.

(ii) investigate the best control strategy for a safe and stable placement of surgical instruments on soft tissue during the tool placement.

Touch interactions and physical contacts are critical factors during the manipulation of tissue/objects. Impedance-controlled manipulators allow the natural transmission of the interaction forces with the environment to the user, but robotic mechanical impedance may mask any delicate force arising from the interaction with soft tissues. A non-linear force feedback torque control was designed in order to investigate if augmented haptic perception is a relevant factor during the instrument's placement on the soft tissue with respect to pure visual feedback. The control parameters were optimized on brain-mimicking gelatin phantoms, which were mechanically characterized to quantitatively evaluate the tissue's damage due to the contact with the tool during indentation. The performances of the robotic assistance with and without force feedback augmentation were comparatively evaluated with respect to freehand task executions. The proposed approach was shown to improve the user's skills in performing a stable and safe tool-tissue contact, allowing for hand tremor rejection and 50% reduction of the tissue's indentation.

(iii) preliminarily study the feasibility of the proposed control approaches for brain cortex stimulation procedures. A group of novice and expert neurosurgeons were enrolled to quantitatively and qualitatively

evaluate the performances of the proposed cooperative control schema (singularly and in combination) in a realistic scenario with brain-mimicking phantoms. The proposed control criteria resulted in comparable performances with respect to state-of-the-art admittance schema with fixed parameters, in terms of pointing accuracy and tissue's indentation overshooting rejection, allowing for the accurate, stable and safe contact with the soft tissue. At the same time, the user efforts during the guidance were reduced by more than 60%.

All the developed controllers were tested in the scope of the EU funded project for brain surgery ACTIVE (FP7-ICT-2009-6-270460). This work support the feasibility of the use of a cooperatively controlled manipulator to assist targeting tasks in open-skull neurosurgery and is in line with the actual research trend in medical robotics, which propose devices that are *effective*, *safe*, both

for the patient and the clinical staff in the operating room, and at the same time that provide the surgeon with interfaces as *intuitive* and *familiar* as possible, in order to reduce the training period and facilitate the acceptance of the technology in clinics.



2. The experimental setup.

ENGINEERED MICRO AND MACROSCALE HUMAN 3D VASCULARIZED BONE MODELS FOR THE STUDY OF ORGANOTYPIC BREAST CANCER METASTASES

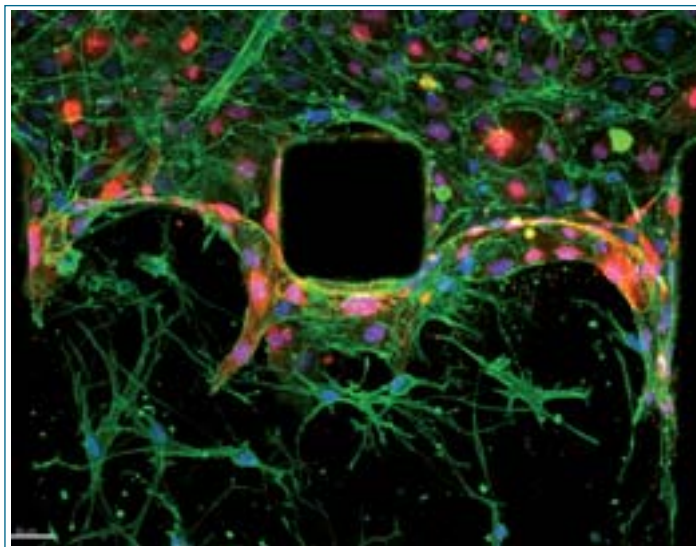
Simone Bersini - Advisors: **Prof. Gabriele Dubini; Dr. Matteo Moretti**

The World Health Organization reports that 14.1 million new cancer cases were diagnosed in 2012 while 8.2 million patients died in the same year. Noteworthy, the spread of primary tumors towards distant organs and the subsequent metastatic colonization is responsible for 90% of cancer-associated mortality. However, despite great advances in basic cancer molecular and cell biology with the discovery of oncogenes and tumor suppressor mechanisms, much remains to be learned about the metastatic process. The cancer biology seed-and-soil paradigm recognizes the existence of organ-specific patterns of metastatization which drive the spread of selected primary tumors towards specific secondary loci. However, despite efforts to model organotypic microenvironments, the organ-specificity of cancer metastases still needs to be elucidated. Then, a deeper understanding of the metastatic cascade and particularly the extravasation process could promote the development of new therapeutics, thus improving cancer survival rates. Particularly, breast cancer is the most frequent cancer among women and the second cause of cancer death in women in more developed regions after lung cancer. Disseminated tumor cells

have been reported in the bone of 30%-40% of early stage breast cancer patients while 70% of advanced breast cancer patients are affected by skeletal metastases, leading to pain, due to spinal cord compression and fractures, and often death. So far, *in vivo* and *ex vivo* models have been developed to study the extravasation process of cancer cells in mice and zebrafish embryos through intravital microscopy. However, they cannot model all aspects of the interaction and cross-talk between human cancer cells, human endothelial cells and human tissue parenchyma. Moreover, strictly regulated, reproducible parametric studies are difficult to perform. Microfluidics can provide useful model systems to investigate complex phenomena under combination of multiple controllable biochemical and biophysical microenvironments coupled with high resolution real time imaging, thus overcoming limitations of traditional assays, e.g. Boyden chamber, which are characterized by limited imaging capabilities and do not provide tight control over the local environment. Up to now, the application of microfluidic techniques to model cancer metastases and particularly extravasation events has been generally limited to the study of chemotactic events, while

no organotypic model has been developed to investigate this key step of the metastatic cascade. However, it is noteworthy to highlight that despite the above mentioned advantages brought by microfluidic approaches, the extremely limited number of cells makes technically hard to perform genetic analyses to investigate the transcription level of key regulatory genes. The present doctoral thesis is focused on the design and optimization of micro and macroscale models to study the organ-specific breast cancer cell metastatization towards the bone. Particularly, a 3D microfluidic model of a bone-mimicking microenvironment surrounded by an engineered microvessel (Fig. 1) was developed to quantify human breast cancer cell extravasation rate, migration distance and micrometastasis generation within the colonized microenvironment, and to highlight the involvement of the CXCL5/CXCR2 pathway in the organotypic extravasation process. Furthermore, a physiologically-like microfluidic 3D model was designed to investigate human breast cancer cell extravasation into bone- and muscle-mimicking microenvironments through perfusable, functional human microvascular networks composed of endothelial and

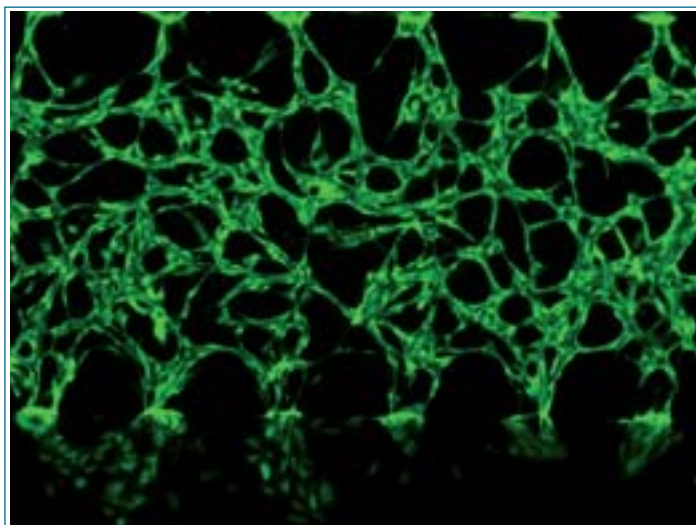
supportive mural-like cells (Fig. 2). This microfluidic model reproduces the pro- and anti-metastatic properties of the microenvironments and provides insights into the different features of organotypic endothelia. The relevance of the present work lies in the application of complex models to investigate and subsequently influence a specific step of the metastatic cascade within different organ-specific microenvironments with critical implications for the development of new drugs, thus fostering a more effective screening of tailored anti-cancer therapies in the context of personalized medicine. Furthermore, a relevant aspect of this doctoral thesis is represented by the design and optimization of human 3D macroscale models of vascularized bone-mimicking tissues through the identification of the optimal combination of experimental parameters leading to the generation of functional vascularized environments, which can be employed to study breast cancer organ-specific metastases by means of post-genomic analyses. Finally, an innovative approach combining microfabrication techniques and self-assembly of vascular structures is discussed in details. Particularly, the main advantages of this approach based on electrochemical cell detachment are the possibility to organize endothelial cells into geometrically defined structures and to produce vessels aligned within micrometric distances in a spatially controlled manner. The unique combination of micro and macroscale 3D models offers a new perspective through which to increase



1. Confocal microscopy of the bone-mimicking microenvironment generated within a microfluidic device. A monolayer of red fluorescent protein (RFP)-transfected human endothelial cells covers the top media channel and the interface with the bone-mimicking channel embedding osteo-differentiated human mesenchymal stem cells within a collagen gel. The dark square represents a post separating two gel regions of the microfluidic device. Red: endothelial cells. Green: F-actin. Blue: cell nuclei.

our knowledge of cancer mechanobiology and investigate key molecular pathways involved in organotypic metastases

within physiologically-like environments, bridging the gap between traditional *in vitro* assays and *in vivo* models.



2. Fluorescence microscopy of a physiologically-like microvascular network. Microvessels connect each other into highly branched microvascular trees generating a complex microvascular network spanning the entire gel channel. Endothelial cells are transfected with green fluorescent protein (GFP).

A FRAMEWORK FOR INTRA-MODALITY IMAGE FUSION APPLIED TO CARDIAC MAGNETIC RESONANCE IMAGING OF THE LEFT VENTRICLE AND 3D TRANSESOPHAGEAL ECHOCARDIOGRAPHY OF THE DESCENDING AORTA

Maria Chiara Carminati - Advisor: **Prof. Enrico G. Caiani**

Recent advances in imaging technology have enabled the non-invasive study of the structure and the function of the heart, the valves and the vascular system.

Different techniques, such as magnetic resonance imaging (MRI), ultrasound (US), computed tomography (CT), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are imaging modalities currently used in cardiovascular medicine and each of them provides specific and complementary diagnostic and prognostic information.

Among these, cardiac MRI and three-dimensional (3D) echocardiography have gained popularity in the clinical scenario, because of their advantages over ionizing or invasive techniques, allowing to assess both anatomy and function of the cardiovascular system. In particular, Cardiac Magnetic Resonance (CMR) imaging is the single modality capable of noninvasively defining cardiac anatomy and function, myocardial perfusion, myocardial viability, and coronary artery anatomy, through the application of different acquisition protocols. From the wide set of MR acquisition techniques, cardiac dynamics can be characterized by cine and tagging CMR, allowing

to track myocardial material points through the cardiac cycle, while the employment contrast-enhanced sequences provides imaging the presence and extent of nonviable tissue in the myocardium, thus revealing its structural impairment.

Three-dimensional echocardiography currently represents a major diagnostic tool in clinical cardiology allowing real-time imaging of the cardiac dynamics. In this scenario, real-time 3D transesophageal echocardiography (TEE) has become one of the most useful imaging modalities for intraoperative management of patients undergoing cardiac surgery. Furthermore, 3D TEE can be employed to acquire images of the aorta, due to its anatomical proximity when introduced in the esophagus, allowing to characterize and quantify aortic lesions, which are known risk factor for severe complications such as stroke and peripheral embolic events.

This PhD work represents a contribution towards the development of procedures for the joint analysis of cardiovascular images. The aim of the project was focused on the development of comprehensive frameworks for the combined analysis of intra-modal information coming from the main non-invasive

and widely used cardiovascular imaging techniques, i.e. CMR and 3D echocardiography.

Two specific contributions, each focused singularly on CMR or 3D echocardiography, are presented. In the first, methods for the 3D assessment of the functionality and the anatomy of the left ventricle are proposed by analyzing and combining the cine and late Gadolinium enhancement (LGE) CMR images, acquired in the clinical routine.

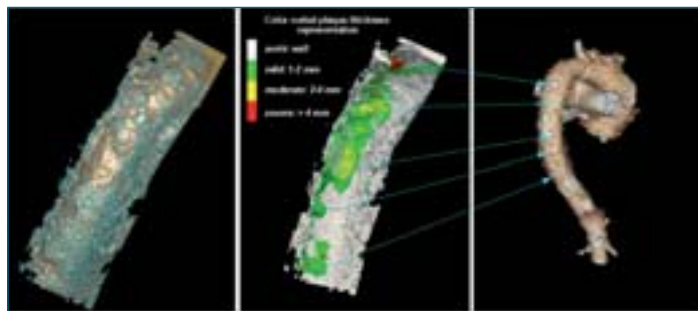
Cine and LGE CMR images are first processed individually to extract relevant information. Cine images were processed to compensate for breath-related inter-slice misalignments, due to the non-exact repeatability of the breath-hold position during acquisition. Then, a 3D ASM was adopted to segment the endocardium by simultaneously analyzing images belonging to the short-axis image stack. To this end, a shape model of the left ventricle was constructed from a large database of semi-automatically segmented 3D echo images, constituted by 205 patients with various pathologies. Left-ventricular wall motion was derived from the 3D endocardial segmentation obtained as the displacement of the endocardium from diastole to systole.

Late-Gadolinium enhanced CMR images were processed to

create a 3D anatomical model of the scar and compute its local transmural. The information derived from the two CMR acquisition is finally combined in the same reference system by a dedicated registration pipeline featuring affine and deformable registration. The described tool allows for the joint three-dimensional analysis of myocardial local function from cine CMR and myocardial viability from LGE CMR images, in a common and patient-specific reference system. This combined information is of established importance for the diagnosis and treatment of cardiomyopathies, allowing to distinguish between reversible and irreversible injured myocardium. Surgical procedures such as revascularization or resynchronization strategies potentially benefit from the knowledge of the exact location of these regions within the LV, as they are significantly related to the likelihood of improvement of contractility after surgery. In the second contribution of the thesis, the employment of real-time 3D transesophageal echocardiography (TEE) is investigated in its ability to image the aorta. The identification and characterization of aortic lesions is recognized to be clinically relevant, as the presence of aortic plaques is an independent

risk factor for stroke and peripheral embolization, being also associated with carotid, coronary and renal artery disease. TEE technology is a suitable tool for assessing aortic atherosclerosis, being routinely performed on patients to identify cardiac sources of emboli and during cardiac surgery to guide the introduction of the cannula into the aorta to prevent peri-procedural plaque embolization. In this scenario, a comprehensive procedure for the reconstruction of the descending thoracic aorta from contiguous 3D TEE images is proposed. First, an ad-hoc image acquisition protocol was designed to acquire spatially ordered and partially overlapped 3D TEE datasets, followed by dedicated image processing to align and fuse all acquired datasets. Alignment strategy implemented pair-wise rigid registration guided by *a priori* knowledge and it was validated using artificially misaligned

images. Image fusion was finally performed to enable visualization and analysis of extended field-of-view of the acquired aorta. The application of different fusion techniques was investigated. The method was applied to a population of 17 consecutive patients. Qualitative and quantitative results demonstrated the potential feasibility and accuracy of the proposed approach. In a clinical scenario, its application could allow quantitative assessment of aortic total plaque burden from 3D TEE images. In conclusion, the design and the experimental application of comprehensive frameworks for cardiovascular image fusion obtained with non-invasive modalities have been studied. The described methodologies may have an effective clinical impact to improve the clinical diagnosis and the definition therapeutic or surgical strategies, as well as for patient-specific modeling purpose.



FATIGUE ANALYSIS OF NITINOL CARDIOVASCULAR DEVICES

Elena Dordoni - Supervisor: Prof. Giancarlo Pennati

It is widely recognized that the assessment of cyclic fatigue resistance is of primary importance during the design process of medical implantable devices made by Nitinol. Focusing on peripheral stents and transcatheter heart valves, due to the presence of cyclic loads imposed by leg movements, as well as by the blood pulsatility, the fatigue resistance is a critical issue since they can experience from 10 up to 40 million loading cycles each year. Therefore, these devices need to be designed to survive at least 10^8 fatigue cycles without failure over the lifetime of the patient, since their fracture could cause the risk of the surrounding tissue damage, as well as the loss in the mechanical properties of the stent-frame itself. This finding highlights the importance of developing a shared, robust and efficient methodology to face the assessment of Nitinol cardiovascular devices fatigue resistance. Therefore, the purpose of this thesis is to face this issue deepening the several involved aspects, in order to give proper guidelines that can be useful both in a design and development phase of a new device and during the assessment of its proper functionality. The main hypothesis is that the numerical analyses or the experimental

tests alone, are useful but not enough to properly assess the Nitinol cardiovascular devices fatigue behavior. Several studies proved that numerical models are a valuable tool to assess the fatigue performance of cardiovascular devices, especially for comparative purposes. The common trend followed by authors is to assign Nitinol material properties taken from literature and to compare the obtained numerical results to a general Nitinol fatigue limit, found in literature. However, the correctness of numerical results strongly depends on the reliability of the material parameters implemented in numerical constitutive models, particularly when the aim of numerical analyses is to provide information about specific stent's risk of fracture associated to a defined loading condition. Moreover, the strong dependence of Nitinol fatigue behavior from all the manufacturing steps, makes mandatory the definition of the material fatigue limit specific for the device under study. On the other side, fatigue *in vitro* tests represent an accepted way to demonstrate a device durability, reproducing as close as possible the actual operating conditions. They are often governed by international standards. Despite the

immediate evidence of safety or failure given by the experimental tests, different disadvantages must be noted: an high number of specimens must be tested to ensure statistical confidence in the results, making the experimental campaigns expensive and time-consuming; difficulty in reproducing the real *in vivo* environment that makes usually experimental tests simplified; difficulty in assessment of biomechanical quantities, since fatigue tests give only the final result (safety of failure), providing in few cases the number of cycles, without any information about the state of stress through the device. In this thesis particular attention is firstly paid to the material properties knowledge, specific for each device under study, since the parameters describing the stress/strain relationship and the fatigue properties are strongly depending on the device dimensions as well as on the whole treatments (thermal and surface finishing) subjected by the device during the manufacturing process. The proposed way to get device-specific Nitinol material parameters to be used as input for material subroutines, as well as to obtain the material fatigue limit for a defined number of cycles, is to perform experimental static and cyclic tensile tests on *ad hoc* material

specimens (e.g. with “dog bone” shape), having cross-section dimensions similar to the device ones and subjected to the same manufacturing treatments. These methods ensure the equivalence between specimens and real device mechanical behavior, allowing therefore to perform experimental material characterization in an easy way. In case *ad hoc* specimens are not available, a methodology to obtain Nitinol parameters from experimental tests carried out directly on the devices is also proposed: the experimental test is numerically reproduced and material parameters are tuned up to reach a good fitting between numerical and experimental testing results. Then, numerical fatigue FEA on peripheral stent models and transcatheter aortic valve are proposed, paying particular attention in properly reproducing boundary conditions: interaction with a confining wall and amount of cyclic load undergone by the stent on one side; pressure pulsatility and effect of leaflet cyclic movement on the valve stent-frame on the other side. Thanks to their reduced costs and high flexibility in modifying geometrical features, boundary conditions and external loadings, FEA revealed a useful tool to prove the main factors influencing fatigue resistance of the studied devices, giving also important indications about how to perform *in vitro* cyclic tests. Numerical analyses on peripheral stents suggest that the *oversizing* due to the presence of a confining wall and its shape and stiffness has to be taken into account during experimental tests for fatigue assessment, while the pulsatile

load can be neglected and the specific cyclic loading condition type (axial compression or bending) due to leg movements may have different influences depending on the stent position along the peripheral arteries. Similarly, fatigue FEA on aortic valve prove that inner pressure and leaflet reaction forces acting on the stent-frame during diastole are the most severe loading conditions and the size and stiffness of the surrounding wall has a strong influence on the fatigue response. Since the fatigue analysis is a very time-consuming process, an additional purpose of the present thesis is to give reliable indications about a device fatigue behavior within a reasonable time: a simplified FE model focusing the fatigue analysis not on the whole stent but just on a unit of interest, is set up and coupled with an analytical model representative of the SFA, which allows to quantify the actual load experienced by the unit associated to particular anatomical conditions. The proposed simplified methodology for fatigue investigation, once applied to a patient-specific case, showed an agreement between model predictions and clinical evidence proved by 18-months follow up data. Despite the approximations introduced into the proposed method, it reveals able to assess the stent's fatigue behavior associated to particular anatomical conditions in a reduced period of time, compatible with the requirements of manufacturers or clinicians. In conclusion, numerical analysis can be a useful tool to

investigate the fatigue behavior of Nitinol devices supporting also the experimental activity, since it can suggest conditions to be tested, evaluating first the device's fatigue response in several possible configurations. However, confidence in the numerical models is only possible after the validation of their results against experimental evidence. For that purpose, experimental tests on real devices are performed in order to validate numerical models predictions: tensile and crimping tests on peripheral stents proves the model capability to reproduce the macroscopic mechanical behavior of the real devices. Similarly, numerical results of crimping tests on aortic valve and experimental evidences are in agreement in proving that crimping procedure induces plasticization in different points of the valve's stent-frame. Finally, an assessment of the proposed fatigue criterion is given by experimental cyclic tests on real stents, which results are found to be in agreement with the proposed fatigue limit.

MODEL-BASED ANALYSIS OF DIFFUSION MAGNETIC RESONANCE:

Study of Microstructural Damage in White Matter and Gray Matter Diseases

Matteo Figini - Supervisors: **Prof. Giuseppe Baselli, Dr. Alberto Bizzi, Prof. Daniel Alexander, Dr. Hui Zhang** - Tutor: **Prof. Alberto Redaelli**

Diffusion MRI (dMRI), an MRI technique sensitive to the diffusive motions of water molecules, has demonstrated good sensitivity to microstructural changes in many diseases. An application is tractography, the virtual reconstruction of fiber trajectories.

Conventional dMRI methods such as Diffusion Tensor Imaging (DTI) rely on the hypothesis of free or hindered diffusion, which is generally a good approximation only at low diffusion weightings (b-values). Many advanced dMRI methods have been proposed; in particular, multi-compartment models of hindered and restricted diffusion in compartments with known geometry were developed to allow a more specific microstructural characterization of tissues.

The aim of this thesis was to assess the feasibility of model-based dMRI techniques in clinical research, and to investigate their utility in the characterization of brain microstructural alterations.

Model-based analysis of dMRI signal in CJD patients

In the first application, mathematical models were developed and applied to study the microstructural changes in Creutzfeldt-Jakob Disease (CJD), the most common human prion disease.

The presence of asymmetric

hyperintense regions on dMRI is a common marker for the diagnosis of CJD, but its origin is currently unknown. Two isotropic bi-compartment models of diffusion were developed to test two neuropathological hypotheses: 1) a biexponential model to describe intra- and extra-cellular hindered diffusion, the latter expected to reduce with protein deposition; 2) a model with restricted diffusion in a spherical compartment modeling restriction in vacuoles. dMRI data were acquired from 10 patients with CJD and 7 healthy and pathological controls. The two proposed models were fitted to the data and regions of interest (ROIs) were delineated in gray matter areas. The fitting performance of both the bi-compartment models was significantly better than the mono-exponential model, especially in the affected areas, but similar among them. In hyperintense areas, the main results were an increase of T_2 , a decrease of all the diffusivities and an increase of the volume fraction of the restricted compartment in the vacuole model.

This study may represent an important step towards the characterization of microstructural changes in CJD. Even though the precise pathological mechanism responsible for dMRI

hyperintensity could not be determined, biomarkers for sensitive and specific CJD diagnosis were proposed.

Microstructural features of brain tumors by NODDI

In the second application, dMRI data from 71 patients with brain gliomas were analyzed with NODDI, a model with 3 compartments where diffusion is free (CSF), hindered (extracellular) and restricted in sets of "sticks" (intracellular), respectively.

A preliminary comparison with an isotropic model showed that NODDI intracellular fraction (f_{ICV}) is a valuable index of diffusion restriction, even though overestimated in isotropic conditions.

Grade II lesions displayed high extracellular volume fractions (f_{ECV}), grade III gliomas showed also regions with an increased f_{ICV} and grade IV gliomas were usually heterogeneous. Instead DTI parameters, namely FA, were non-specifically altered.

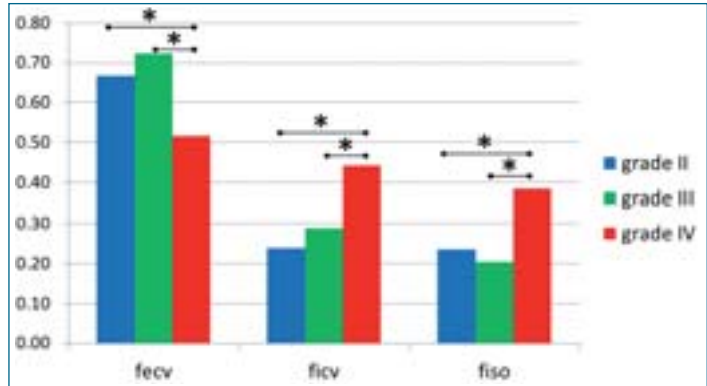
All the three NODDI volume fractions allowed a statistically significant discrimination between grade IV and both grade II and grade III lesions (figure 1).

These preliminary results show that non-invasive tumor characterization and grading by NODDI is feasible in a clinical context. This could have

an important impact on the preoperative evaluation of brain tumors.

NODDI-based tractography in peritumoral edema

In a last application, an algorithm for tractography based on NODDI parameters (NODDIT) was developed and applied in 10 patients with glioblastoma multiforme (GBM) to test the possibility of a better reconstruction through areas of vasogenic edema than allowed by DTI-based tractography (DTT). In the proposed NODDIT algorithm the termination criterion was based on upper thresholds on both the orientation dispersion index (ODI) and on f_{ISO} . In a preliminary phase, the ODI threshold was calibrated by comparison with DTT in healthy regions. The mean streamline density obtained by NODDIT and DTT was evaluated in 3 ROIs per patient: the tumor core, the peritumoral edema and the contralateral WM. Compared to DTT, NODDIT streamline densities were similarly high in normally appearing WM, similarly very low in the tumor core, and significantly higher in



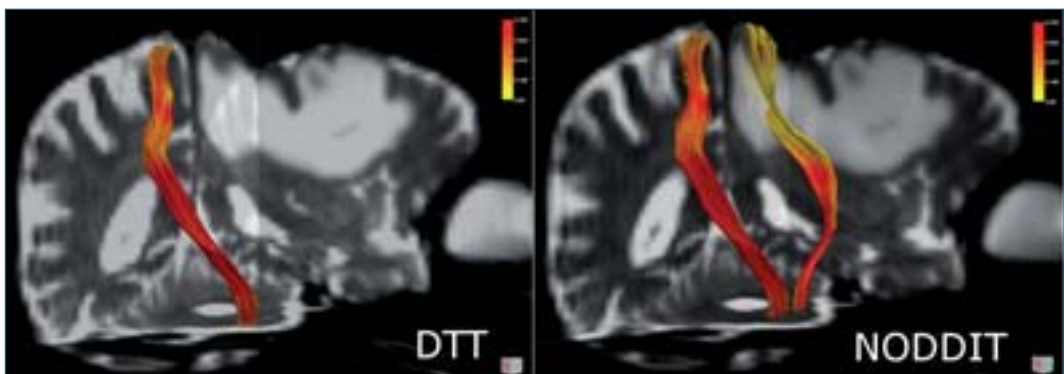
1. Mean f_{ECV} , maximum f_{ICV} and f_{ISO} averaged among the patients with grade II, grade III and grade IV gliomas. The significant differences are marked with stars.

vasogenic edemas. Lowering FA threshold to about 0.1 can provide similar results to NODDIT in the edemas; however, an unacceptable specificity loss was highlighted, with a high number of false positives, as verified even in the ventricles. The visual inspection of the tractographic reconstruction of specific tracts showed that in all the considered cases NODDIT provided more streamlines passing through the edemas, or even allowed reconstructing tracts completely missed by DTT (figure 2). Thus, NODDIT could find

important applications in the preoperative mapping of patients with brain gliomas.

Conclusions

In all the proposed applications, the application of multi-compartment models was clinically feasible and advantageous when compared to traditional methods. This involves a non-trivial work for the choice of suitable models and the interpretation of results, but the obtained parameters seem more specific to the underlying tissue microstructure and its pathological changes.



2. Example of tractographic reconstruction in a representative GBM patient. The cortico-spinal tract was reconstructed from the same ROIs with DTT (A) and NODDIT (B).

PECTIN HYDROGELS FOR REGENERATIVE MEDICINE: INJECTABLE SYSTEMS, CELL DELIVERY AND ANTIMICROBIAL FORMULATIONS

Roberta Gentilini - Advisors: **Prof. Maria Cristina Tanzi, Dott. Paola Petrini**

In the last years, novel hydrogel formulations were developed to obtain injectable products for tissue regeneration. The advantages of using injectable hydrogels rely on their ability to conform to the defect shape and on the possibility of in vivo delivery in a minimally invasive way, thus reducing discomfort and complications for the patient. As the challenging strategy implies that the cells are loaded inside the gels. For cell-loaded injectable gels, cell entrapment is generally achieved in the liquid (or highly viscous liquid) form of the gel precursors, with severe limitations on the conditions and reagents used in the sol-gel transition often not compatible with cell viability and bioactive molecules immobilization. Pectin, a natural polysaccharide present in the cell wall of most plants, is nowadays object of increasing interest for applications in the biomedical field. Pectin is a biocompatible anionic polysaccharide that constitutes 30% of plants wall [1], widely used as thickener, gelling agent, stabilizer and emulsifier in food products [2]. It is almost entirely composed of three polysaccharidic domains: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RGII). HGA is the major component of pectic polysaccharides and

contains α -(1 \rightarrow 4)-D-linked galacturonic acids (1.4- α -D-GalA) that are partially methyl-esterified and sometimes partially acetylated. Due to the peculiar gelling mechanism, low methoxy pectins, which have a degree of esterification (DE) < 50, have been proposed for the preparation of hydrogels for biomedical applications, namely drug delivery, gene delivery and regenerative medicine as implantable material for minimally invasive surgery. Pectin gels are proving wide applicability as biomaterials with recent advances in regenerative medicine application, such as microspheres. Bioactive modifications, such as enzymatic degradation, partial oxidation and RGD functionalization of this polysaccharide were able to control degradation and cell adhesion. Hairy regions of branched pectin, separated by enzyme degradation, are known to promote proliferation and differentiation of cells (such as BMSCs to osteoblasts). In view to obtain an ad hoc pectin-based hydrogel for a specific purpose, pectin can be extracted from different sources and its characteristics vary according to the plant species from which it is extracted. The main characteristics of the appropriate process of extraction are the use of biocompatible chemicals and the possibility to

preserve the peculiar structural characteristics such as the integrity of branched regions, which show an important role in cell interaction. A high molecular weight and a low degree of esterification need to be pursued to form stable, ionotropic gels, in compatible conditions with cell viability or biomolecules loading.

In view of developing pectin-based injectable systems for cell immobilization in applications of regenerative medicine, the results of this work demonstrated that the gelling kinetics and rheological properties of pectin hydrogels can be modulated according to the specific way of administration and to the tissue to be treated. Particularly, by fine tuning of sodium bicarbonate and calcium carbonate content, a tight control of the pH of the hydrogel solutions was achieved, thus controlling their gelling kinetics. In this context, it was possible to obtain hydrogels with fast gelation that can be used as in situ gelling systems, or gels with a slower gelation process that can be used as cell carriers, where the gel preparation is performed prior to injection. Furthermore, the control of the rheological parameters allowed obtaining thicker hydrogels to tailor the mechanical stimuli of the matrix and promote cell differentiation

for different tissues, providing a three-dimensional structural support for the host cells. Cell immobilization within a hydrogel represents an innovative and successful strategy to deliver cells in a damaged tissue. It is well known that cells remain viable into injectable microspheres, where the surface area to volume ratio is higher and the exchange of nutrients and oxygen is promoted. In this thesis, the process for adipose-derived stem cell (hADSC) immobilization in pectin-based bulk hydrogels was investigated. hADSCs retained viability up to 24 hours after immobilization within the 3D pectin matrices and the presence of glucose and glutamine as additives resulted to play a key role in nutrient and oxygen supply during the first hours of immobilization, which are known to be the critical phase for entrapped cells. After extrusion, the injectable hydrogels showed an excellent hADSCs viability, indicating that the presence of a 3D matrix protects cells from the damaging injection process. Immobilized hADSCs maintained their stemness capability after 7 days of incubation, indicating that the hydrogel did not affect the cell phenotype.

To provide an ideal environment for anchorage-dependent cells, a peptide grafting of pectin is

a strategy often investigated in literature. In this thesis, a peptide-grafting was successfully tested on pectin backbone to improve antibacterial activity. Eventually, antimicrobial pectin hydrogels will be useful tools in several diseases, such as osteomyelitis, in which the optimal treatment raises from the dual approach of cell delivery associated to antibacterial effect, with the intention to induce bactericidal effects and bone regeneration in a single step. To these aims, we demonstrated that it is possible to produce a pectin derivative endowed with antimicrobial activity by grafting antimicrobial peptides on the pectin backbone.

Considering the specific final aim, i.e. biomedical application, ad hoc production of pectin should be explored with tailored extraction process, able to preserve its peculiar structural properties and with the possibility to form ionotropic gels. These hydrogels could be evaluated as three-dimensional (3D) cell culture systems, where the cells can be entrapped during gel formation in mild conditions. The 3D systems can be produced as injectable stem cell-loaded hydrogel for tissue regeneration, by controlling the biochemical environment within the gel. Injectable systems are not limited to cell delivery, and can be exploited to release

specific biomolecules or to be modified for facing a specific challenge. The antibacterial injectable pectin hydrogels can be used in multiple applications where preventing bacterial adhesion is still an unmet need. This research is aimed to the ultimate goal of combining these different aspects producing a contactive material composed of different substances, both effective to dismantle biofilms, and deliver cells in the damaged tissue, disrupted by the infection, for pathologies such as osteomyelitis and periodontitis.

PATIENT-SPECIFIC MULTI-PARAMETRIC MODEL OF THE HEART FROM MDCT IMAGES TO GUIDE VENTRICULAR Tachycardia ablation procedures

Sofia Goncalves Antunes – Supervisors: Giovanna Rizzo, Sergio Cerutti

In the pre-procedural planning and guidance of electroanatomic mapping (EAM) and radiofrequency (RF) catheter ablation (CA) procedures in ventricular tachycardia (VT), the knowledge of the exact location and extent of myocardial scar is important. Today, delayed enhanced magnetic resonance imaging (DE-MRI) is considered the imaging gold standard for the assessment of scar tissue and it is being used, integrated into the 3D EAM system, to guide ablation procedures. However, multi-detector computed tomography (MDCT) could be an interesting alternative. The main reasons are related to the reduced artefacts caused by the implantable cardioverter-defibrillator (ICD), the higher spatial resolution when compared to DE-MRI with which we can have detailed information about the anatomy of the heart (e.g. trabeculae and coronary arteries) as well as the reliability in visualizing epicardial fat distribution. The purpose of this work was to construct a 3D multi-parametric model of the heart by segmenting automatically ventricular cavities, left myocardium, myocardial scar, epicardial fat and coronaries from MDCT images. Further, this map was compared with the findings of EAM, created previously to the RF CA procedure.

Cardiac Anatomy Segmentation

Image-guidance allows the navigation of the catheter tip over the cardiac structures of interest within a high resolution anatomical map. For this intent, the accurate segmentation of the patient specific cardiac anatomy of interest is a fundamental effort. Of great importance in any epicardial intervention is epicardial fat tissue, it may be confused with myocardial scar causing useless ablation. Moreover, the anatomical course of the main coronary vessels may help in optimizing epicardial interventions, reducing potential damage of such structures. For the anatomical segmentation a 3D level set algorithm based on a multi-scale directional stopping function was developed, implementing the Geodesic active contour (GAC) formulation. The stopping function was applied twice updated after the convergence of the first evolution, reducing the scale space of the edge detector, until the level set converged again. We validated the proposed method quantitatively on the with the manual segmentations done by expert radiologists.

Myocardial Scar Segmentation

There are three different

approaches to detect non-viable myocardium including perfusion defects in MDCT; two approaches are on the early angiographic scan and one on the delayed scan. On the early scan compromised zones are hypo-enhanced when compared to the normal myocardium or present myocardial wall thinning. The approach to detect scar on the delayed scan, contrary to the first case, consists in searching for hyper-enhanced zones of the myocardium. We took the advantage of having the myocardium segmentation from the early scan, where the myocardium boundary is clearly defined, to use as template for the scar identification. Since the heart cannot be seen as rigid body, we model the transformation as a sum of global rigid transformation and a local elastic deformation (free-form deformations) for the correction of the deformations that may occur due to the beating heart and other anatomical motions. The extraction of nonviable myocardium was accomplished as described in the following. In the case of hypoenhancement, we calculated mean μ and variance σ^2 of the entire data and used as higher threshold $\mu - 3\sigma$ to identify the low attenuation values. In order to accurately measure myocardial wall

thickness, the papillary muscles within the LVendo structure need to be excluded from the structure. Due to the 3D bending geometry of the LV cavity, we used the alpha-shape. Then, from each LVendo triangle vertex, a ray was casted outwards, and the distance to the nearest point on the LVepi surface was taken as the myocardium wall thickness. Points with distance less than 5 mm were considered scared areas.

As the detection of myocardial scar areas in the delayed scan consist in detecting high density values when compared to the normal myocardial tissue, we studied different thresholds to detect scar as similar as physicians would do. We made different assumptions to determine the best way to identify automatically areas of scar; the first one was that scar in DE MDCT has similar attenuation values than the blood pool. For this reason, using the LVend segmentation from the early scan as template on the pre-registered delayed scan, we identified the parameters of the Gaussian distribution of the LV attenuation values (μ, σ). In order to identify the best parameters to identify scar, we used as thresholds the following values: μ , $\mu - 1\sigma$ and $\mu - 2\sigma$.

Looking to the myocardium, on the other hand, as it involves (if scar is present) a mixture of two tissue types (corresponding to two different histogram attenuation peaks), a Gaussian mixture model with two component densities was used to identify two clusters on the myocardial tissue. It consists

in a parametric probability density function described as a weighted sum of two Gaussian densities $g(x|\mu_i, \sigma_i)$. We obtained mean μ and variance σ values of the two Gaussians. To identify hyperenhanced areas other thresholds were also μ and $\mu + (\mu - \mu)/2$ experimented (healthy myocardial tissue mean value μ_h and nonviable myocardial tissue mean value μ_p). The best threshold to detect DE scar in our experiment was the formulation: $\mu + (\mu - \mu)/2$, which best matched with the experts identification of hyper enhanced zones. Using this threshold, it was possible to extract scar most in agreement with the opinion of the physicians on the scar location, with a satisfactory classification.

Myocardial Multi-Parametric Map

Using information from the automatically segmented cardiac structures of interest, describing scar location and extent, as well as zones of thick fat layers we face the myocardial map construction. Additionally, the created map was compared with the findings of EAM, created previously to the CA procedure. The decision of an endocardial or epicardial EAM and RF ablation approach was taken basing on the prevalent distribution of scars at CE-MDCT or during the failed endocardial procedure. Values greater than 1.5 mV defined normal LVendo bipolar electrogram amplitudes and values greater than of 8 mV defined normal LV endocardial unipolar electrogram amplitudes. 9 patients have an ICD; 8 had only an endocardial and one only an epicardial intervention, and two patients

had both an endocardial and epicardial intervention (and consequently a EAM).

To construct the mesh of the myocardium, containing information about the myocardial wall thickness, epicardial fat depth and myocardial scar areas, we extracted a triangulation (surface mesh) for each segmented structure. Using the distances from the LVepi to the LVendo surface, zones where the thickness was less than 5 mm were considered scar vertices. For each triangle vertex of the LVepi mesh, the distance from LVepi to the epicardial fat surface was computed; points above 3 mm were considered myocardial scar.

In patients that underwent an epicardial intervention, points with epicardial fat thickness greater than 3mm were added to the myocardial mesh to be considered as myocardial scar. Bipolar and Unipolar voltages were correlated with MDCT based scar 35.3% of low bipolar voltages of the EAM, and 64.72% of unipolar low voltages points are within our defined scar.

Conclusions

This work represent a step forward not only to the accomplishment of accurate patient-specific segmentation of cardiac structures, but also to the introduction of MDCT scar detection as a feasible and effective approach to plan EAM, aiming at improving CA RF and reducing intervention time.

PRECLINICAL EVALUATION OF POSTERIOR SPINAL FIXATORS – CRITICAL ASSESSMENT OF THE CURRENT INTERNATIONAL STANDARDS AND SUGGESTIONS FOR IMPROVEMENT

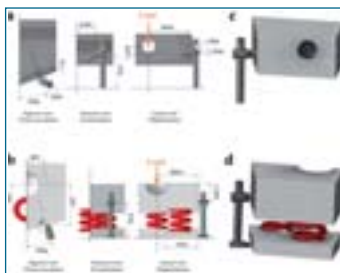
Luigi La Barbera - Relatore: Tomaso Maria Tobia Villa

Posterior spinal stabilization by means of pedicle screw-rod based implant is a gold standard in the surgical treatment of a great variety of diseases. Clinical results demonstrate an improved outcome with respect to traditional techniques stand alone. Significant improvements have been made since the introduction of pedicle screw technology in 1980-90's, however a high complication rate is reported still today (from 12.0 to 54.0% after 2000). Moreover, fatigue-related failures, due to the high number of loading cycles experimented during everyday life activities (e.g. walking), are continuously reported especially at the screw level (from 1.2 up to 35.0% after 2000). The present dissertation is aimed to better understand and possibly improve the current standards published by the American Society of Testing and Materials (ASTM) and the International Standardization Society (ISO) for the *in vitro* preclinical evaluation of the mechanical behaviour of posterior spinal stabilization devices. In particular, the validity of ASTM F1717 and ISO 12189 standards, representing a vertebrectomy (*worst case*) and a physiological instrumented 2-Functional Spine Units (FSUs) scenarios respectively (Figure 1), was investigated.

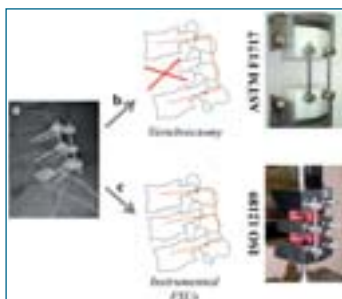
Anatomical (e.g. pedicle inclination, interpedicular distance), as well as biomechanical parameters (e.g. follower load distance, center of rotation distance, unsupported screw length), useful to catch the most important features of the thoracolumbar spine, were collected as a function of the spinal level both from a review of the literature and using direct measurements on physiologic subjects. A parametric FE (finite element) analysis was performed on both standards evaluating the contribution of each parameter in increasing the stress arising on the implant (Figure 2). A few parameters were found to be significant (i.e. leading to a percentage stress increase greater than 2%). Moreover, the geometrical configuration implemented in ASTM F1717 standard reproduces quite well an average instrumented thoracolumbar spine segment; however the anatomical worst case scenario due to the combination of the most important anatomical parameters was found at L1, leading to a maximum stress increase of about 15% for ASTM F1717 and about 10% for ISO 12189 standards: these scenarios were then proposed as a basis for a revision proposal of the standards. Other mechanical parameters, such as the initial precompression of the anterior

support as well as its stiffness in ISO 12189 standard, were found to be significant, leading to a much higher stress increase (even beyond 420%) than the anatomical parameters alone: in this light, care should be taken when using ISO test method. Since the above described results were obtained on a simplified design capable of catching only the general features of a pedicle screw-rod based implant, a more realistic design was also considered. A parametric FE model of a commercial spinal fixator was built and validated using strain gauges technique. A parallel preliminary experimental mechanical characterization revealed that the fatigue life of constructs assembled according to the proposed revised version of ASTM standard may be significantly lower than those assembled according to the current one: this was interpreted as a confirmation that a standard revision should be taken into consideration. A final numerical analysis was led to better understand whether the stress arising on the posterior spinal stabilization device in standard configurations may be really representative of some everyday life activities. A previously validated L2-L4 spine segment was then instrumented according different clinical scenarios: vertebrectomy, bisegmental

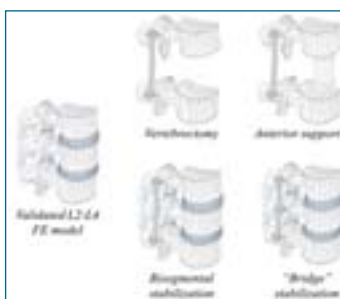
stabilization, bisegmental "bridge" stabilization and vertebrectomy with an anterior support (Figure 3). These models were validated against *in vitro/in vivo* literature data. The results demonstrated that ISO 12189 procedure reproduces quite well a physiological instrumented scenario during flexion of the upper body. Moreover, simple considerations can help in comparing and interpreting the achieved results using different testing procedures with respect to the effective clinical use.



2. Parametric FE models according to ASTM F1717 and ISO 12189 standards (a, b respectively) and corresponding meshed reference models (c, d respectively).



1. Sagittal view of a patient instrumented with a spinal fixator to stabilize the lumbosacral segment (a). Simplified drawings of the experimental approaches used to evaluate the mechanical properties of posterior spinal fixators according to a vertebrectomy scenario implemented by ASTM F1717 standard (b) or to a physiological instrumented scenario according to ISO 12189 standards (c).



3. Starting from a validated L2-L4 FE model, different clinical scenarios were simulated. The vertebrectomy scenario was directly compared with ASTM standard model, while the bisegmental stabilization, bisegmental bridge stabilization and a vertebrectomy with an anterior support were compared to ISO 12189 models where the spring stiffness was also varied.

MULTIMODAL MAGNETIC RESONANCE IMAGING AT 3 T AND CHALLENGES FOR APPLICATION AT ULTRA-HIGH FIELD

Eleonora Maggioni - Advisors: **Prof. Anna Maria Bianchi, Prof. Sergio Cerutti, Ing. Gianluigi Reni**

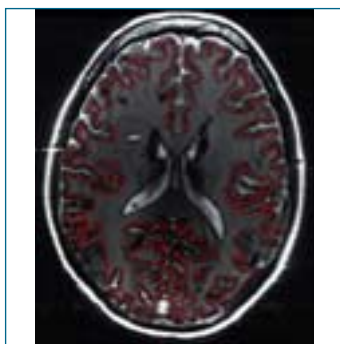
Multimodal neuroimaging opens up a unique window of opportunity for the investigation of function and structure of the brain. Each imaging method probes specific physiological processes with characteristic resolutions, giving a filtered view on one or more brain processes of interest. The combination of different imaging modalities can overcome the limitations of the single techniques, as it allows to 1) extend the coverage of the spatiotemporal domain and 2) get a more comprehensive view of physical and physiological properties of the brain. The present PhD dissertation gives a comprehensive overview of the advantages and possibilities provided by brain magnetic resonance imaging and its integration with complementary neuroimaging techniques, with special interest towards simultaneous EEG-fMRI. Indeed, the integration of EEG and fMRI offers the unique opportunity of providing a non-invasive comprehensive view of brain function with high temporal and spatial resolution. The primary objective of the PhD thesis is to develop technical instruments for pre-processing, analysis, coregistration and fusion of multimodal information, to be used at normal or high magnetic field strengths. Multimodal imaging at ultra-high field (UHF) offers

enormous opportunities that are currently unfulfilled, due to important technical challenges: in this respect, the PhD thesis focuses on the pre-processing phase to set the ground for future complex unimodal and multimodal analysis at UHF. The first section deals with the pre-processing of EEG-fMRI data and is dedicated to the removal of cardiac-related artefacts from EEG data recorded in MR environment at 3T and 9.4T. Using resting state data, the performances of different sophisticated correction methods based on independent component analysis (ICA) were quantitatively compared, in terms of their capability to 1) reduce the artefact, 2) recover the underlying physiological information. Since the discrimination of artefactual components from physiological ones is challenging, different methods for the selection of PA-related components were considered. In the 3T study, the selection based on the components wavelets transform, which is a novelty of the thesis, resulted the most accurate in preserving the physiological alpha rhythm in the occipital channels. In the 9.4T study, the overall EEG physiological information was distorted by the UHF, from amplitude and spectral content to connectivity pattern of EEG

data. For the first time, using the new selection method based on wavelets, we have been able to recover at the channel level the physiological alpha rhythm in the occipital channels. Although the quality of removal can be still largely improved, these preliminary results pave the way for future resting state EEG-fMRI analysis at UHF. In the second part of the thesis, the interest is shifted towards the processing phase. Methods for complex fMRI connectivity analysis are described: in particular, a novel whole-brain parcellation scheme that integrates MRI anatomical and functional information is presented. The new parcellation divides the brain into non-overlapping spatially connected clusters and can be used to define nodes in connectivity networks that are homogeneous in both structure and function. The test on two synthetic datasets demonstrated an overall capability of the algorithm to correctly identify the functional clusters, both in resting state and in presence of stimulations. The reliability of the novel method was further confirmed by real data applications: the parcellation 1) showed a good reproducibility across healthy subjects, 2) led to a reliable definition of epileptic networks. To set the stage for future

connectivity analysis at UHF, the delicate issue of anatomical segmentation at 7T is introduced: besides higher problems of inhomogeneity, the tissue contrasts exploited at UHF can be different from the most common ones and may require specific processing techniques. The Tissue Border Enhancement (TBE) technique allows an immediate visualization of the borders of brain tissues: a new algorithm for the extraction of borders in TBE images is described, called Minimum Intensity Snake Algorithm (MISA). MISA follows iteratively the path of minimum intensity within the image using functions of graph theory. When applied to a TBE image acquired at 7T, it led to a satisfactory detection of tissue interfaces (Fig.1). The combination of TBE and MISA can overcome the limitations related to traditional imaging techniques at UHF, opening the road to several applications. In the last section of the thesis, a set of methods for the analysis and integration of EEG, fMRI and NIRS data is shown. A comprehensive overview of the information of clinical utility that can be extracted from the EEG and fMRI techniques is given, as applied to the study of photosensitivity. The effects of intermittent photic stimulation (IPS) on one patient were compared with a group

of healthy subjects. The fMRI response to IPS was investigated, then the EEG information was used to study evoked potentials, frequency content and functional connectivity in the IPS frequencies. An EEG-informed fMRI analysis investigated the hemodynamic correlates of the EEG power changes in the IPS frequencies. The quantitative comparison between patient and control group revealed many peculiar characteristics that contributed to delineate the patient's clinical picture. Finally, in the patient, the fMRI epileptic network was extracted and the pattern of propagation of the epileptic activity within the network was inferred.

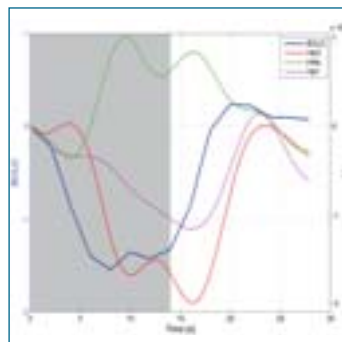


1. Borders between GM and WM identified by MISA.

In a group of healthy subjects, the negative BOLD responses (NBRs) to IPS were investigated by means of the NIRS technique, able to give insight into the

BOLD determinants. The results of the fMRI activation analysis were compared to the NIRS ones and the coupling between BOLD and NIRS signals was investigated in the NBR regions. NBRs were found to be characterized by an HbO decrease and a concomitant HHb increase w.r.t. baseline condition (Fig.2): the NIRS study provided new information on the negative BOLD phenomenon.

In summary, the present PhD thesis has given insight into some crucial analytic challenges of the multimodal integration at normal and high field, drawing attention on the combination of MRI with other neuroimaging techniques, especially EEG. The results are promising and open the door to future complex multimodal analysis at UHF.



2. Plot of BOLD and hemoglobin responses to IPS in one example channel (channel 3-4) of one exemplar subject. The grey area corresponds to the IPS interval.

MONITORING OF VITAL PARAMETERS FOR SLEEP ASSESSMENT THROUGH WEARABLE AND SMART DEVICES AN APPLICATION FOR MOOD STATE CLASSIFICATION IN BIPOLAR PATIENTS

Matteo Migliorini - Advisors: **Profs. Annamaria Bianchi, Sergio Cerutti**

Quality of sleep is then one of the aspects that mostly influence our everyday life. Literature report that a high percentage of serious car or work accidents are caused by daytime somnolence due poor sleep quality. It is important to remark that the difficulty in the identification of symptoms and the costs connected to an accurate clinical evaluation can give rise to underestimation or to the lack of diagnosis of sleep disturbances. In addition, generally, there is a small number of specialized centers. Further, a bad quality of sleep was proven to have an impact on blood pressure, decreases the immunity defenses and may increase the insurgence probability of metabolic disturbances such as obesity and diabetes. In addition, sleep disturbances, for example, the ones related to breathing, have a strong association with cardiovascular pathologies. For all these reasons, an indication on the sleep quality may constitute a good parameter for prevention of some pathologies and can provide a tool for improving quality of life. The introduction of textile wearable devices, as well as sensorized mattresses constitutes a great advantage in prevention and also in follow-up. In fact, they can be used at home without the intervention of medical personnel. Thus,

a home monitoring system could represent a cost-effective solution for continuous monitoring and, therefore, risk prevention.

Sleep is also a sensitive barometer of emotional status and psychiatric conditions. Sleep difficulties have been associated with emotional states and psychiatric diagnoses related to anxiety, affective dysregulation (depression, manic states and bipolar disorders), post-traumatic-stress disorder and other more specific behavioral disorders such as attention deficit and hyper-activity disorder (ADHD). The evaluation of a sleep disorder is often mixed with the assessment or consideration of the psychiatric condition. Hypersomnia may be the main symptom in some depressive disorders, as seasonal depression, depression with atypical features or depressive episodes in bipolar disorder. Psychological state assessment, in particular bipolar disorder management, is one of the areas of great demand for the need of continuous monitoring, patient participation and medical prediction. The nature of bipolar disorder is unpredictable and episodic. Thus, it is necessary to take the traditional standard procedures of mood assessment through the administration of rating scales and questionnaires, and

to integrate this with tangible data found in emerging research on central and peripheral changes in brain function that may be associated to the clinical status and response to treatment throughout the course of mood disorders. Disease management for psychiatric patient, through continuous, non-invasive monitoring represents a novel approach. Today, psychiatric patients together with their relatives face a great deal of problems, resulting often in a premature interruption of treatment and of follow-up by psychiatric services, due to the deinstitutionalization of mental health services and the establishment of services in primary care, community centers and general hospitals. The use of wearable devices can open the possibility to provide continuous and ubiquitous access to medical excellence in a cost-effective way.

Sleep is a complex state physiologically characterized by important changes in the autonomic regulation of the cardiovascular activity. HRV is largely affected during sleep by sleep-stage organization: specifically, evidence suggests a predominant vagal drive to the heart and a reduced sympathetic tone during non-rapid eye movement (NREM) sleep and an increased sympathetic modulation, with fluctuations

between parasympathetic and sympathetic influences, during rapid eye movement (REM) sleep.

The new generation of technological tools, in particular in the field of telecommunication, material science and digital signal processing, gave rise to wide application of wearable and smart devices for monitoring vital signs (in particular HRV signal) in ambulatory subjects during their daily activities. These devices allow the remote and continuous monitoring of people in different circumstances and situations, such as diagnosis procedure, monitoring of patients with cardio-respiratory or mental diseases. This is because the patient lives his/her daily life and is not perturbed psychologically by the hospital environment. This also results in economic savings by the reduction in hospitalization costs.

This PhD work aimed to give a mathematical characterization of sleep, both to provide a clear and scientific quantitative description of the underlying physio-pathological phenomena, and to allow the implementation of an automatic classifier for speeding up the study of sleep macrostructure in regular clinical practice. Moreover, it aimed to characterize the regulatory autonomic behaviors behind the course of the bipolar disorder in order to pave the way for the creation of a supporting tool to help clinicians in facing better this disease.

It was demonstrated that the HRV signals contain information that can be connected to the sleep modulations that can be inferred from time-domain,

frequency-domain and long-term regularity parameters. Using the information present in literature, four different classifiers were trained obtaining results that can be considered comparable in terms of both accuracy and value of K. What it is interesting to notice is the reliability in estimating a parameter of clinical interest like the sleep efficiency (SEFF). The dissertation aims to elevate the attention on evaluating sleep classification performance by means of sleep-related features (like SEFF, TWT, WASO, etc...) instead of the highly used metrics, i.e. accuracy, sensitive and specificity. These sleep-related features find application in clinical studies; therefore, it seems more useful to estimate them in a reliable way than computing a precise hypnogram. The HRV and the sleep regulations influence different pathologies including mood disorders. Some changes in modulation of the ANS was demonstrated to be amenable to bipolar disorder, while other processes reflect physiological mood changes in bipolar patients.

The crucial point of such analysis was represented by the choice of monitoring the patients in a naturalistic environment. The study of a psychiatric disorder during the real life, at patient's home gives results of relevant clinical importance. On the other hand, such uncontrolled environment might be affecting the recording quality. It was shown a possible way to face this issue through two steps. First, by evaluating the minimal requirements for a reliable estimation of the considered features. In particular, the

robustness of the estimation of the regularity parameters has been studied as function of the amount of data. Secondly, implementing the requirements in a series of rules to decide, recording-by-recording, which feature satisfy them. Following this procedure, some features can be calculated even if the requirements for computing the others are not satisfied. For example, if the ECG recording is completely corrupted, macrostructure-related sleep parameters (TIB, SOL, WASO, TWT, TST and SEFF) can still be estimated through the analysis of the body movement signal. In this dissertation was demonstrated the feasibility of a home monitoring of bipolar patients. The information that can be gathered from the HRV signals seemed to be useful for the assessment of the different stages of such pathology. Moreover, they represent an objective evaluation of the disease providing instruments for its study and interpretation. This can be taken into consideration from the clinicians and the caregivers to better take care and support the patients.

A NOVEL IMAGE REGISTRATION STRATEGY FOR ONCOLOGICAL PEDIATRIC BRAIN IMAGES FUSION

Eros Montin - Advisor: **Prof Luca T. Mainardi** - Tutor: **Prof Paolo G. Ravazzani**

Image registration is one of the fundamental procedures of image processing in the field of medical imaging. Medical images are widely used for diagnosis, treatment planning, disease monitoring and image guided surgery and can be acquired using different imaging modalities like Magnetic Resonance Imaging (MRI), Computed Tomography (CT), X-ray, Positron Emission Tomography (PET). Images obtained using different modalities, usually, need to be compared to one another and/or combined for analysis and decision making. In this scenario the aim of image registration is to find the best alignment between a fixed (reference) image and a moving one (source) by evaluating their similarity. Image registration can be visualized as an optimization problem which maximizes the matching between these images by changing the parameters of a geometrical transformation which maps points in one image to the corresponding points in the other one.

An image registration procedure is mainly composed by three components: a geometric transformation model, a similarity measure and an optimization algorithm. Due to the diversity of images to be registered and the diverse

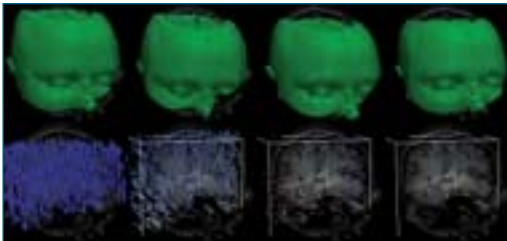
types of degradations, it is impossible to design a universal method applicable to all the fusion tasks. Hence, in order to obtain an optimal solution, a greater attention has to be paid during the selection of the above components which have to be defined according to the nature of the specific registration problems. Among these three components an important role is played by the metric. Intensity based fusion metrics assume that some particular features extracted by image voxels will be most similar when the correct registration transform is applied. They can be sub-divided in two macro groups in relation to the kind of registration task they solve: mono-modal and multi-modal. The first one solve registration task of images acquired with similar parameter settings, conversely, multi-modal one solve all the others.

Therefore, one of the most challenging problem in the image fusion field arises from multi-modal images registration. Literature metrics based on information theoretic techniques had great experimental success and are becoming widely used in the multi-modality fusion activity. Among them, Mutual Information (MI) is considered the elective state-of-the-art. However, MI optimization is still considered a hard task

because of the several well-known drawbacks e.g. the non-convexity of the metric in the parameter space. Therefore, registration strategies based on MI are usually constrained in order to obtain smooth deformation fields. This deformation smoothness is not always desirable in oncological field where the lesion presence may induce huge localized tissues changes. Therefore in this thesis, an alternative similarity measure has been proposed and implemented. The metric integrates MI with a local descriptor of the images to be matched in a pluri-metric approach. Since two images are considered fused when intensity changes occur in the same location in the two images to be registered, the elected local descriptor was a gradient based filter. The reliability of this metric was evaluated in comparison to the literature ones and finally utilized in a real clinical oncological case.

The main aim of this study was the reassessment of images acquired in pediatric age in comparison to the adolescent one, by means of Diffusion Tensor Imaging (DTI). The activity concerned the late realignments of images arising from different scanners, modalities, patient age and therapies. The main challenges of this task arise from

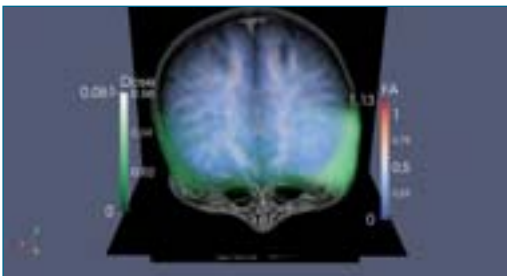
both the classical registration problems (such as patients movement during the scans) and the growth of the patient during these evaluations which could be also more the 9 years.



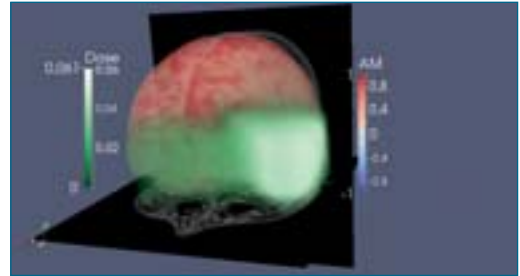
Longitudinal image registration of pediatric MRI, by increasing the complexity of the transformation model using a pluri-metric approach. Namely translation, rotation, scaling, and non-rigid based on b-spline.



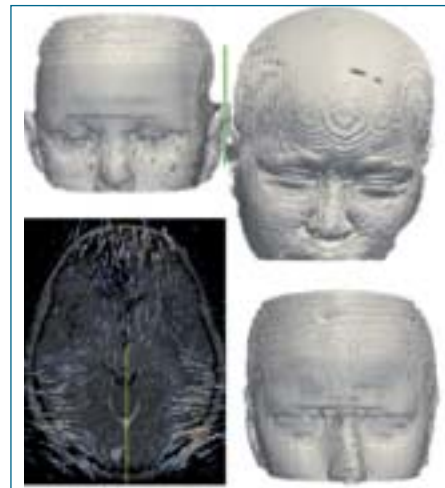
The neuro-radiological atlas exploited in the atlas-based segmentation, the colored area represent a part of the regions considered in the segmentation. In red is also reported the dose of a patient as example.



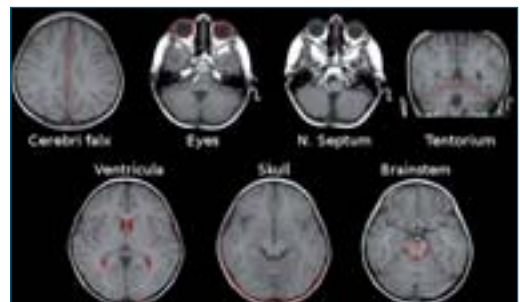
Fusion of radiotherapy and diffusional dataset (FA).



Fusion of radiotherapy and diffusional dataset (AM).



MRI0 (bottom right corner) creation from the MRI1 (top right corner) using a deformation field (bottom left corner) which mimic a real patient growth pattern. MRI 0 can be compared with the real MRI0 (top left corner) of the patient.



The seven anatomical areas used as focus point during the registration performance assessment of the registration between CT and MRI0.

ADVANCED MICROFLUIDIC PLATFORMS FOR INVESTIGATING THE ROLE OF MICROENVIRONMENTAL SIGNALING ON STEM CELL FATE

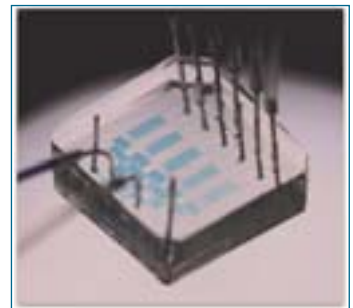
Paola Occhetta - Tutors: Prof. Alberto Redaelli - Ing. Marco Rasponi

In vivo, cells are surrounded by a complex multi-factorial environment characterized by specific physicochemical properties (temperature, pH, oxygen tension), which provide cells with exogenous stimuli deriving from soluble factors, cell-matrix interactions, and cell-cell contacts. The orchestrated and spatio-temporally dynamic interplay of these biochemical and physical extracellular cues, referred to as *cell microenvironment*, regulates cells structure, function and behavior, ultimately guiding their fate. Considering the complexity of the native cell microenvironment, *in vitro* models are required as tools for better understanding the key pathways regulating cells behavior. In order to achieve reliable and biologically relevant results, such models should feature the ability to *in vitro* recapitulate the dynamic combinatorial role of soluble factors, matrix-bound cues, cell-cell contacts and cell-matrix adhesions on cell responses. To date, much of the current understanding in cell biology relies on traditional bi-dimensional (2D) *in vitro* cell culture models, which mainly consist in the static culture of cells seeded on polystyrene flat surface plates (mm to cm in characteristic dimension). Such substrates, however,

poorly mimic the native cellular microenvironment, featuring levels of stiffness that are orders of magnitude higher than those found *in vivo* and lacking in presenting 3D cues typical of the native cell physical environment. In the past few decades, several attempts have been made to address these issues proposing macroscale 3D culture models relying on innovative biomaterials, often combined with laboratory-scale bioreactors. Although providing a more reproducible and controlled approach to investigate mechanisms of cell behavior within a 3D environment and to engineer functional constructs, these approaches still have to deal with size-scales that are orders of magnitude bigger than the native microenvironment. Recently, microscale and microfluidic technologies are finding increasing applications as innovative approaches in cell biology studies, enabling an unprecedented control over the cellular microenvironment while reducing the time and the scale of experimental platforms for better matching the cellular level. Moreover, they allow for automating and parallelizing experimentations and coupling cell cultures directly with high-throughput analysis systems, thus improving simultaneously model accuracy

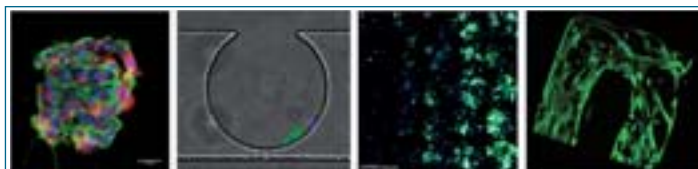
and experimental throughput. Considering these premises, microfluidic platforms represent promising *in vitro* models, increasingly exploited as enabling tools in the field of cell biology, from the screening of drugs or molecules, to the optimization of culture conditions for inducing specific cell fates.

In this scenario, this PhD project envisions the exploitation of the main principles of microfluidics for the generation of innovative technological solutions for addressing specific questions in the field of cell biology.



1. The main principles of microfluidics were exploited for the generation of innovative technological solutions for addressing specific questions in the field of cell biology.

The aim of this research is thus the development of microfluidic platforms and techniques as tools for investigating and modeling the effect of different cues from the cellular microenvironment in addressing



2. Four microscale platforms and/or techniques are presented. For each platform, a specific microfluidic strategy has been applied to define a technological solution for addressing a specific biological goal.

stem cell fate. In details, four microscale platforms and/or techniques, ad hoc conceived in the context of national and international collaborations, are presented. Each chapter is focused on a single platform and underlines how a specific microfluidic strategy has been applied to the definition of a technological solution for addressing a specific biological goal.

The first presented microfluidic platform was designed with the aim to (i) generate and culture 3D cellular microaggregates under continuous flow perfusion while (ii) conditioning them with different combinations/concentrations of soluble factors. An exploitation of the platform is proposed, in collaboration with the Tissue Engineering Laboratory (University Hospital of Basel, Switzerland), to perform studies on limb bud development and investigate processes involved in mesenchymal progenitor cells differentiation, towards a 'developmental engineering'

approach for skeletal tissue regeneration.

A second microscale strategy is introduced to spatially tailor the 3D microenvironment around cells, based on the combination of an innovative biocompatible photopolymerizable hydrogel (VA-086-GelMA) and an easy to handle photo-mold-patterning (PMP) technique. The work presented in this chapter is partially the result of a collaboration with the Cell and Tissue Engineering Laboratory (IRCCS Galeazzi Orthopedic Institute, Milano, Italy).

As third technique, a microfluidic cell mixer is presented, as the result of a collaboration with the Tissue Engineering and Microfluidics Laboratory (TEaM, University of Queensland, Australia). The integration of this mixer as upstream functional element within two different microfluidic platforms is then demonstrated for the automatic establishment of 2D and 3D osteogenic co-culture models, aiming at investigating the influence of pre-osteoblastic cells

on human mesenchymal stromal cell osteogenic commitment. Finally, a fourth microfluidic platform is introduced for (i) trapping and culturing single cells into defined spatial configurations, (ii) while automatically delivering them concentration patterns of non-diffusive particles (i.e. gene vectors). An exploitation of the platform is then proposed to perform on chip high-throughput screening and optimization of transfection strategies, in the context of a collaboration with the Biocompatibility and Cell culture Laboratory (BioCell, Politecnico di Milano, Italy).

AUTOMATIC DECELLULARIZATION AND DECELLULARIZED SCAFFOLDS FOR BLOOD VESSELS TISSUE ENGINEERING

Alessandro Filippo Pellegata - Advisor: **Prof.ssa Sara Mantero**

Introduction

Decellularization is the complete removal of all cellular and nuclear material from a tissue preserving its extracellular matrix. The results of the process are scaffolds that have biochemical properties able to stimulate the cell adhesion, proliferation and differentiation beside to mechanical properties similar to the native tissue and a preserved tissue structure. To date, the majority of decellularization processes are performed with manual operation, limiting the safety, reliability and reproducibility of the process while these are mandatory requisites in the translation to the clinic. To overcome this issue the automation of the process can fulfill the previously stated requisites and some groups are now performing decellularization using devices. Decellularized scaffolds have been used also for blood vessels tissue engineering trying to fill the existing clinical gap for small caliber arterial substitutions. Decellularized vascular scaffolds have been investigated in various in-vivo model and reached also two cases of clinical implantation but for large vessels substitution. However it is not yet clear in the blood vessels tissue engineering field, and also for decellularized vascular scaffolds, if it is needed an endothelialization previous

to the implantation or if the host body could be used as both cell source and bioreactor with positive outcomes. Considering this complex scenarios, this thesis aimed at the development and characterization of a decellularized arterial scaffold and its characterization in-vitro and in-vivo. Beside this, in order to overcome the limitations of manual operated processes this thesis aimed also at the development of a device for the automatic decellularization of blood vessels that could autonomously drive the process while keeping sterility and while being easy and versatile to use.

Results

A device able to drive a whole automated decellularization process for blood vessels was designed and developed. It consists of an hydraulic system able to perfuse and recirculate up to three decellularization solution, a thermal regulation system and an user interface. The chamber was designed ad-hoc for the application, perfusion is provided by means of dedicate holders, the system can house vessels of different lengths (up to 100 mm) and diameters (3-7 mm), an innovative feature of the device is the distal holder whose weight generates a longitudinal strain on the vessel and, at the same time, the internal geometry gives

a pressure drop that coupled with a pulsatile pattern of the pump exerts a circumferential strain on the vessel. The system can be programmed to drive complex protocols that involve different decellularization solutions repeatedly exchanged according to user-defined patterns, the process parameters that can be specified are the number of solutions, the timings, the recirculation pattern, the flow rates and the temperatures. Sterility and reliability of the device were validated.

The swine arteries were decellularized using the automatic device with hypotonic-detergent-serum protocol in order to validate both protocol outcomes and device functionality. Results showed no residual cells nor nuclear material. Mechanical properties testing showed no statistically significant differences in respect to native tissue for both compliance and burst pressure. Moreover outcomes showed that the mechanical stimulation enhances the decellularization in case of 3 mm diameter vessels. The evaluation of the decellularization of swine arteries using the hypotonic-detergent-enzymatic protocols resulted in a good degree of cell removal and preserved ECM structure with good mechanical

properties. HE staining, DAPI and DNA quantification assessed the absence of cellular or nuclear material while HE, Movat Pentachrome and SEM observation confirmed the maintenance of ECM structure. The mechanical characterization revealed no statistically significant differences. Vessels implanted in the chimeric human swine model explanted at 2 weeks were patent with vWF+ cells covering the lumen and an ongoing migration of α -SMA+ cells from the adventitia to the media. The 10 weeks trial showed a more spread and deep repopulation of the vessel wall by α -SMA+ cells. Interestingly at 2 weeks it was observed that no cell positive for human CD31 was present in the lumen while the cells forming the tunica intima were positive for swine CD31 meaning that seeded cells were lost and substituted by host cells. On the contrary, results of the unseeded implants showed that except for the 6 weeks explant, all the other time points resulted in occlusion because of the growth of amorphous fibrous tissue inside the lumen and intimal hyperplasia as reported by HE staining. The occlusion was populated by α -SMA+ cells, new vessels, lipids deposition and sparse macrophages. The investigation of macrophages interaction with decellularized scaffolds showed that decellularized scaffolds elicit a lower expression of genes for IL-1 β , IL-6, MMP9, TNF- α at 24h compared to silk scaffolds while after 96h only IL1 β and IL6 were lower. Arg I expression was instead higher at both 24 and 96h, CD206 was higher at 24h. VEGFA expression showed

no relevant differences, however it was observed an increasing trend for silk and a decreasing one for decellularized. ELISA assay showed that macrophages on decellularized scaffolds do not produce IL-6 opposite to those seeded on silk fibroin.

Discussion

The decellularization protocols presented in this thesis brought positive decellularization results as cell removal and preservation of the ECM mechanical properties, moreover the hypotonic-detergent-serum protocol allowed to limit timings and costs. The cell removal and the mechanical properties are crucial for blood vessel tissue engineering because possible cellular remnants could elicit high inflammatory response or calcification, while a compliance mismatch could elicit intimal hyperplasia. Storage is an unavoidable step in the whole process of scaffold production and it was also demonstrated that the -80°C storage do not affect the mechanical properties. The device developed proved to be functional and effective for the decellularization of blood vessels, and it represents an approach aimed at the automation of the whole process rather than a tool to provide a mean of decellularization, like perfusion. Furthermore the device showed an enhancement for the decellularization of very small caliber vessels thanks to the innovative system of perfusion that provides radial and longitudinal strains with a simple setup. The chimeric human swine in-vivo implants resulted in patent vessels both at 2 and 10 weeks. Interestingly it was demonstrated that the

endothelial cells seeded are lost at 2 weeks and substituted by host cells confirming the data reported in the literature for both small and large animals models. On the contrary, unseeded implants ranging from 6 to 14 weeks occluded due to intimal hyperplasia and growth of fibrous tissue in the lumen. These results end in the still open question on the need of an endothelialization previous to grafting, indeed the literature reports opposite data and there is a contradiction between the loss of seeded cells and the better outcomes of seeded grafts. Our findings have the peculiarity of the absence of antiplatelet drugs administration while this therapy is usually provided. Overall, a supposition can be made on a paracrine role in the early phases for endothelial cells that influences the late term resolution of the implant. To give preliminary insight on these phenomena we analyzed the macrophages response to decellularized scaffold and results showed a lower and less persistent inflammatory response in comparison to the response to silk fibroin scaffolds.

IN VITRO ELECTROPHYSIOLOGICAL STUDIES OF NEURONAL NETWORKS: A NOVEL DEVICE FOR RELIABLE, PROLONGED AND HIGH THROUGHPUT MICROELECTRODE ARRAY EXPERIMENTS

Giulia Regalia - Supervisors: **Prof. Alessandra Pedrocchi** and **Prof. Giancarlo Ferrigno**

In the field of modern Neuroscience research, cultures of primary central neuronal cells coupled to substrate-integrated Microelectrodes Arrays (MEA) represent an unparalleled methodology to study network-level electrophysiological properties of neuronal ensembles in both physiological and pathological conditions. Nowadays, non-invasive and multisite MEA recordings of neuronal electrical activity are a mainstay technique for studies about neuronal networks dynamics, neuronal plasticity and drug and toxicology tests. Due to the role that results from these studies are expected to play in Neuroscience research, reliability and reproducibility of the experimental outcome are crucial for MEA-based studies. To this aim, a first requirement is the presence of physiological conditions during experiments. However, the recurrent withdrawal of cultures from the cell incubator to perform MEA recordings results in the deflection of environmental parameters (e.g. temperature and gaseous atmosphere composition) from canonical *in vitro* growth conditions, in mechanical stress to the cultures and in increased infection risk. Such perturbations trigger functional alteration and changed cell viability, limiting the duration of single experimental

sessions (i.e. up to 1 hour), which prevents from performing the uninterrupted tracing of neuronal processes that develop over extended periods of time (e.g. several hours to several days or weeks), such as network development, long-term plasticity or effects of chronic pharmacological treatments. A second key requirement is the possibility to perform parallel MEA recording of multiple cultures, which enhances culture-to-culture comparability and shortens the experimental timescale, with an important impact on pharmacological tests. Third, compactness and accessibility to the MEA setup are essential features to allow the integration of technological tools needed to perform experiments (e.g. microscopes, pumps).

Notwithstanding previous efforts to improve standard MEA experimental setups, still an experimental platform is missing that integrates the capabilities to properly meet all the three abovementioned requirements. This work is a technological contribution towards the development of a stand-alone mini-incubator capable of providing uninterrupted MEA data in a high-throughput format while keeping permanently neuronal networks in physiological conditions, thus enhancing reliability and

reproducibility of MEA readouts. Towards the establishment of such a device (Fig. 1), multiple activities have been performed over the PhD work. As a preliminary step, the spontaneous activity of hippocampal cultures (n=96, three different cell densities) was tracked with a standard equipment (i.e. brief recordings once every 48 h) over 1 month *in vitro*, which allowed to obtain statistically robust reference data to validate the device to be developed. Then, a portable environmental chamber was characterized and tested with cell viability assays and it was proven to grow neuronal cultures on the lab bench in a comparable fashion with respect to standard cell incubators. Starting from this proof-of-concept, an advanced prototype was designed, including: (i) a closed and compact chamber housing 4 cultures on MEA; (ii) integrated environmental sensors (relative humidity, carbon dioxide, temperature) coupled to a custom electronic control unit and a real-time data logging software, able to assure incubator-like temporal stability, accuracy and spatial homogeneity; (iii) custom electronic boards compatible with the environmental chamber and capable of reading out neuronal spikes from 4 60-channel MEAs with a signal-

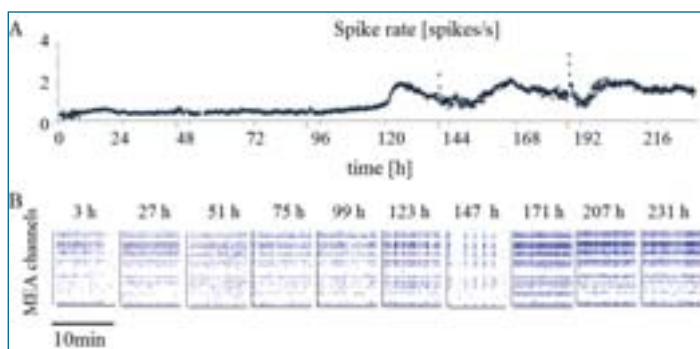
to-noise ratio comparable to standard recording devices; (iv) air-tight external access to chemically manipulate the cultures without withdrawing them from the chamber; (v) a versatile software tool for on-line classification of neuronal spikes detected by MEAs, able to save time in data analysis of prolonged MEA recordings. The prototype was successfully deployed to perform long lasting (from some hours up to 10 days) electrophysiological recordings of neuronal culture spiking activity, demonstrating the possibility to trace the unperturbed evolution of neuronal activity patterns (Fig. 2). Moreover, the validity of the system in performing prolonged and parallel neuro-pharmacological stimulations has been demonstrated by means of reproducible data over prolonged pharmacological dose-response experiments. It can be concluded that the work presented in this Thesis constitutes a valid platform to perform MEA experiments on *in vitro* neuronal cultures under physiological environmental conditions. The design and experimental verification of the system have been thoroughly reported in the Thesis. Overall, the developed device has advantageous capabilities for electrophysiological and pharmacological studies: (i) MEA

recordings with observation continuity, (ii) standard operating conditions of cell culture practice, (iii) avoidance of environmental fluctuations, (iv) reduction of the operator intervention, (v) reduction of the number of replicates and the time required to have significant results, (vi) enhanced culture comparability and data reproducibility (vii) nullification of stress to cells and infection risk during cell culture chemical manipulations (medium change and pharmacological tests) and (viii) easiness to integrate other devices in the system. The device lends itself to be employed in studies of neuronal phenomena such as long-term plasticity, pharmacological dose-response experiments, investigations of chronic effects of pharmacological treatments

and of mechanisms at the basis of late-onset neurodegenerative pathologies mimicked *in vitro* (e.g. Alzheimer's disease).



1. Scheme of the developed experimental platform intended for prolonged and parallel *in vitro* neuronal recordings by means of Microelectrode Arrays (MEA).



2. Example of a prolonged MEA recording (10 days) of a neuronal cell culture inside the devised system, showing the time course of the spontaneous spiking rate averaged across the electrodes (A) and 10-minute snapshots of spike timing at each recording site extracted at different time points (B).

EXPERIMENTAL AND NUMERICAL CHARACTERIZATION OF NATIVE BONE TISSUE AND GLASS CERAMIC BONE SCAFFOLD AT SMALL SCALE

Mohamad Shahgholi Ghahfarokhi - Supervisor: **Prof. Pasquale Vena**

It's every human being's right to live with dignity and the absence of suffering during his/her lifetime, but our lack of knowledge on systemic, organ, tissue and cell degeneration due to senescence indicates that the ageing process is often beyond our control. Therefore, plenty of studies are carried out in different laboratories to expand the related knowledge and amend the treating methods. Most models of aging at cellular level are derived from simple in-vitro experiments in addition to animal models ranging from fish to primates.

Changing in physiological microenvironment and alternations in signaling between cells and even distant tissues and organs leads to systemic age related diseases such as osteoporosis. This project is an initial step towards a long-term goal of generating reliable biomimetic models of a bone tissue. The tissue should be applicable for studies of pathological conditions and development of pharmacological strategies reducing animal and clinical testing as well as time and cost associated with them. As a contribution to this wide framework, this study aims at studying the mechanical properties of the bone tissue and of glass-ceramic scaffold at small length scale by making use of the nanoindentation

technique. In particular, the study of the bone tissue is focused on the dependence of the mechanical properties on the applied load or characteristic size of the experiment. The study was aimed at identifying and quantifying a damage mechanism occurring in the bone tissue upon loading. The experimental characterization was carried out on both the cortical bone tissue as well as on the trabeculae of spongy bovine bone samples. The damage mechanisms were further investigated by means of numerical simulation of the nanoindentation experiments at multiple characteristic lengths on the cortical bone samples. The chosen material for bone scaffolds is a glass-ceramic material derived from a highly bioactive glass (called CEL2) (Vitale-Brovarone et al. 2008). It shows very promising biochemical properties and it can be produced with a controlled multi-scale porosity, which is significantly important to improve the scaffold characteristic. Hence, the goal of this study is to perform a mechanical characterization of the glass-ceramic scaffold at different scales, in particular micro and macro scales. For such a study, a bulk form of the material, non-pores (without any porosity), is examined as well as a 3D Porous material,

in order to run a survey on porosity dependency of the mechanical characteristic of the material. In addition, beside the glass ceramic material, the study went through mechanical characterization of trabecular/cortical bone of bovines at the same scale.

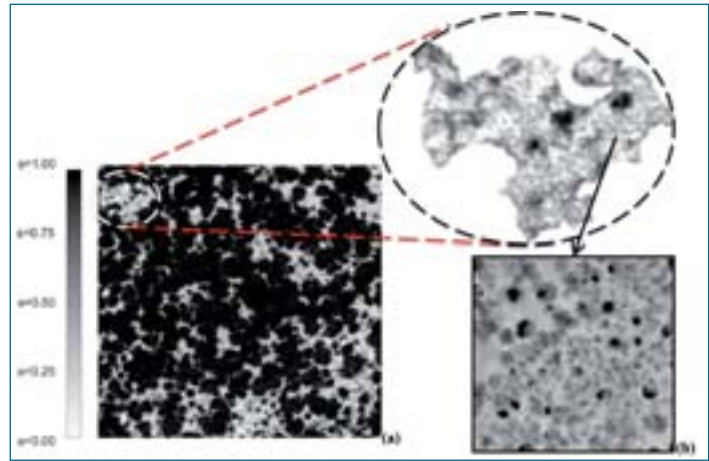
The mechanical properties at the micro scale are investigated by means of the nanoindentation experimental technique and the mechanical properties at the macro scale are obtained by means of computational tools only. The data found through the experiments is used to feed the computational models for the macroscopic characterization. The 3D structure of the scaffold has been scanned with a micro-CT scanner for further investigation. Based on the scanned images, a binary volume is built up, in which the value 1 is indicating the material and the value 0 is indicating porosity. Subsequently, a finite element model based on the binary volume is developed in order to model the structural geometry of the sample and to estimate the mechanical properties of the scaffold at macro scale. The results of the computational model are checked by result of an analytical model. The model was adopted based on an analytical approach proposed by Zhu et al. (1997). This model

is using a simple unit cell to set-up the geometry of the scaffold walls in order to predict the Young's modulus of the structure as a function of the porosity. A good agreement between the results is found confirming that the prediction from the computational model is an effective approach for above-mentioned purpose.

Moreover the computational model results show the anisotropy of the scaffold. This feature is investigated utilizing two different approaches.

First the structure anisotropy is evaluated by the calculation of the *Mean Intercept Length* (Whitehouse 1974), that is a standard method to check if the structure is mainly aligned in a specific direction. A degree of anisotropy of 25-30% is found in this way. On the other hand, the *effective volume* (Quinn 2003) is calculated in order to quantify the unloaded volume, due to inhomogeneity of the stress distribution in the structure during the simulations. According to the results, the structure anisotropy can be explained properly by both anisotropy of the scaffold architecture and inhomogeneity of stress distribution.

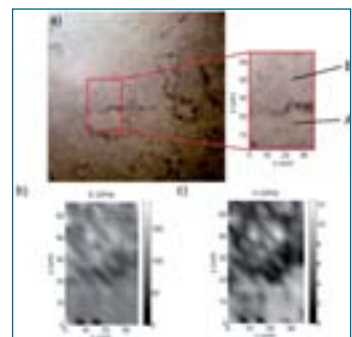
The interpretation of comparison between the mechanical response of the glass ceramic material and that of the bone tissue is that the glass ceramic bulk material does not exhibit the typical damage response (decreasing indentation modulus with increase of indentation load). On the other hand, the decreasing trend of indentation modulus was observed by the same indenting on the 3D sintered ceramic scaffolds walls. The decreasing trend found on



1a. Gray level distribution of the nanoporosity across a layer obtained by μ CT-scans, dark voxels related to high porosity, whereas white voxels relate to low nanoporosity. The image is from an area indicating $9556\mu\text{m} \times 9940\mu\text{m}$ by 696×724 pixels, in which each pixel includes $13.73\mu\text{m} \times 13.73\mu\text{m}$ area.
1b. A schematic view of a pixel size area illustrating both glass-ceramic material and nanoporosity obtained by SEM.

the scaffold walls was owed to the intrinsic porosity of the sintered ceramic. Consistent values with other investigation techniques carried out by the research group in the Polytechnic university of Turin validated the results found in this study. As a general conclusion, the 3D glass-ceramic scaffolds which are designed and manufactured with the final aim to build "tissue models" to be used *in-vitro* testing of drugs simulating healthy, diseased or aged bone tissues have a good potential to achieve their purpose. The manufacturing process to obtain the 3D scaffolds, which should exhibit mechanical and physical properties consistent with those of the trabecular bone in different aging or clinical conditions, can be tuned so to provide the scaffolds with the desired elasticity and strength properties. The experimental and computational framework reported in this study provides

an effective tool to the prediction of the mechanical properties of the scaffold as a function of their physical properties like macro and micro-porosity and 3D architecture.



2. Colorimetric maps of the reduced modulus and hardness related to areas (bulk glass-ceramic)

TUBULAR STRUCTURE TISSUE ENGINEERING: DESIGN AND VALIDATION OF A NOVEL MULTIFUNCTIONAL ROTATING BIOREACTOR

Ilaria Stefani - Supervisors: **Prof. Sara Mantero, Prof. Justin J. Cooper-White**

Injuries, genetic diseases, cancer, ageing: all things that can harm the complex functioning of the human body. In 2012, an estimated 56 million people died worldwide, with noncommunicable diseases (NCDs) responsible for 68% of all deaths globally, up from 60% in 2000, registering an alarming increase.

Cardiovascular disease alone accounted worldwide for 17.5 million deaths in 2012, that is three in every 10 deaths. In terms of proportion of deaths that are due to NCDs, high-income countries have the highest proportion (87%), followed by upper-middle income countries (81%). Ischaemic heart disease, stroke, lower respiratory infections and chronic obstructive lung disease have remained the top major killers during the past decade. End-stage organ failure or tissue loss is also one of the most costly problem in medicine: over 8 million surgical procedures are estimated to be performed every year to treat these disorders in the United States alone, incurring a tremendous health care cost of more than \$400 billion annually. Over the last 50 years, transplantation of an extensive variety of tissues, reconstructive surgical techniques, and replacement with artificial devices have significantly

improved patient follow-up and life expectancy. Despite new surgical techniques and drug improvement, these solutions present many limitations, such as donor shortages, permanent immunosuppressive regimens, increased risk of infection, unwanted side effects, and, in case of artificial supports, finite durability.

This scenario led to an increasing interest in the field of tissue engineering, which merges engineering and life sciences knowledge with the final goal to develop *in vitro* cellularized functional substitutes able to restore or improve tissue and organ activities. The three most important ingredients of tissue engineering are biomaterials, cells collected from a patient and proper environmental culture conditions (i.e., a bioreactor). In summary, a porous delivery system is needed that confines the cells to the desired location, after *in vitro* mechanical stimulation. Significant progress has already been made in the field and examples of successful clinical implants of tissue-engineered products include skin substitutes, nasal cartilage, functioning bladder and trachea. Whilst these are promising results, much effort is still required *in vitro*, to elucidate basic mechanisms regulating cell response, and the behaviour of the engineered

construct during maturation, and *in preclinical models* to investigate the host response (e.g., neovascularization, remodelling), and the behaviour of the produced substitute once grafted.

One area of particular interest is in the replacement of damaged hollow organ structures such as those found throughout the cardiovascular, respiratory, urinary, and gastrointestinal systems. Whilst the current surgical procedures for replacement of damaged tissue commonly use autologous grafts, this is hampered by the poor availability of suitable graft tissue, donor site morbidity, and poor long term stability of the substitute. As such, there is a critical demand for the production of tissue engineered grafts that are capable of meeting the functional requirements of the organ system without inducing immune or inflammatory responses, or losing function over time.

The presented research project, aimed to design, fabricate and characterize an innovative multifunctional bioreactor for the regeneration of hollow organs, able to overcome the limits of the current available devices.

A prototype was produced able to perform rotation of a tubular scaffold along its longitudinal

axis, to allow scaffold tensioning and to connect with different hydraulic circuit in a fast and trustworthy way. Bench test results demonstrated that a reliable and easily assembled device was developed. The main characteristics of the system were: 1) ease of handling, that makes the system user-friendly, and reduces the risk of contamination; 2) versatility of the system; capable of axial rotation, separate media environments for inner and outer layers, and the ability to connect to different hydraulic systems to produce laminar and pulsatile flow within physiologically relevant pressures. 3) compatibility with the best standard of good laboratory practice. With this new system we were able to combine different stimuli and safely join rotation, perfusion and gentle stretching of the cultured patch, all functions that until now were separately considered in dynamic cultures. The preliminary culture experiments, carried out with Biofelt[®], gave the proof of the functionality of the bioreactor. The obtained results revealed that the application of rotation stimuli favoured the penetration of the cells into the scaffold wall, enhancing the production of extracellular matrix. For the testing the bioreactor

potential to produce a tissue engineered tubular grafts, an innovative PCL/PLA-TMC based electrospun tubular scaffold was realized, and chemically and mechanically characterized. The three-dimensional matrix demonstrated mechanical properties comparable with native blood vessel tissue, presenting a promising candidate for vascular tissue regeneration. PCL/PLA-TMC matrix and the developed bioreactor recreated a suitable 3D environment for mesenchymal stem cells growth and differentiation. The results confirmed that 3D dynamic culture allowed for a better control over the cell fate and behaviour by facilitating mass transfer phenomena, by facilitating the medium to flow through the scaffold wall. Moreover, the transmural flow favoured cell migration through the thickness of the tubular matrix, permitting extracellular matrix formation and deposition along all the structure. Whilst the scaffold showed both favourable mechanical properties as well as an outer layer, which facilitated mesenchymal stem cell colonisation for the eventual formation of a tunica media, the ability of the inner layer to support growth of cells that would form a tunica intima was not tested. With further refinement of

scaffold production, creating a multi-layered structure with a smooth continuous layer facing the internal lumen, it is expected that endothelial colonisation of the inner layer will be possible. This would then permit more comprehensive testing within the bioreactor, and a demonstration of the full versatility of the developed system. With the dual chamber organisation of the bioreactor, growth conditions for the development of smooth muscle and endothelial tissue layers can be independently optimised. Furthermore, whilst rotation and double phase culture were demonstrated to be successful, the use of hydraulic pumps to provide pressure, load, and flow stimulation on the tissue can be tested; both in terms of directing the cells to form a suitable tissue for grafts, and in terms of performing rigorous testing of potential grafts. We can state that this research led to the production of both an innovative device for tubular organs regeneration, and the characterisation of a novel scaffold for potential use in vascular grafts. With the modularity of the bioreactor along with a relative ease of use, the device holds great potential for future production of tissue engineered tubular grafts.

IMAGE PROCESSING OF 4D PHASE CONTRAST MRI DATA FOR HEMODYNAMIC AND MORPHOMETRIC ANALYSIS OF THORACIC AORTA

Daniele Tresoldi - Advisor: **Ing. Giovanna Rizzo, Prof. Anna Maria Bianchi**

Tutor: **Prof. Paolo Ravazzani**

Introduction

Cardiovascular diseases (CVDs), a group of disorders that affect the heart and the vessels, are the leading cause of death worldwide. In order to assess CVD initiation and progression, blood flow characteristics are known to play an important role, since hemodynamic alterations are closely related to pathological condition and may have a causative role in CVD evolution. In order to better understand the mechanism of initiation and progression of CVD as well as to assess the presence of the pathology condition, flow patterns study should be integrated with the morphometric characterization, which consists in evaluating size (diameter or radius, area) and shape (curvature or tortuosity) of vessels. In fact, a strictly relation between alteration of blood flow characteristics and changes in morphology of the vessel has been demonstrated in many aortic diseases.

3D cine Phase Contrast MRI (4D PCMRI) can extract a quantitative depiction of spatial distribution of blood flow velocity as function of time together with magnitude images visualizing the subject's anatomy. This imaging technique, based on the observation that spins moving through magnetic field have a phase shift proportional to their velocity, is now

considered the gold standard in research to study the blood flow characteristics evolving in thoracic aorta and it is also used in clinical protocol to extend traditional anatomic evaluation. The aim of this project is to study, to develop and to evaluate advanced methods to allow and to improve the use of 4D PCMRI images of the thoracic aorta for hemodynamic and morphometric evaluations. Two main purposes can be distinguished: 1 - to propose a new filtering approach able to denoise and regularize 4D velocity maps providing volumes suitable for hemodynamic applications and 2 - to develop a new segmentation method to extract the vessel lumen and to create a 3D model which can be directly used to calculate patient-specific indices for a comprehensive morphometric and hemodynamic aorta characterization.

Materials and Methods

The proposed noise reduction strategy is the application of an Anisotropic Diffusion Filter (ADF), a well-known filtering technique able to reduce data noise preserving image contours. ADF is based on the anisotropic diffusion equation, which controls the evolution of a filtering smoothing function through the characteristics of the image, such as the proximity

of edge or constants areas. This approach, after being properly calibrated, was applied on PCMRI images acquired with different SENSE reduction factors and its effects were evaluated in terms of image quality (noise in velocity images), regularity of the velocity fields (divergence of the velocity field, relative error in velocity magnitude and absolute error in flow direction), aorta flow pattern visualization (streamlines, and secondary flow patterns) and flow rate quantification.

To segment thoracic aorta lumen from velocity data, a new approach was developed, which is based on Level Set algorithm a computational technique able to control the evolution of a contour through an implicit function, widely used for its ability to follow the topology change of the object. The vessel of interest was first manually identified by an operator and then a two-step algorithm was applied: an initial rough surface was calculated using a Fast Marching Level Set which was then refined, smoothed and adapted to the local morphological characteristics of the vessel using a Level Set Geodesic Active Contour (GAC) approach. This new method was tested on subjects acquired with and without SENSE parallel imaging and it was validated in terms of area overlap and mean

and maximum contour distance between manual and automatic segmentation.

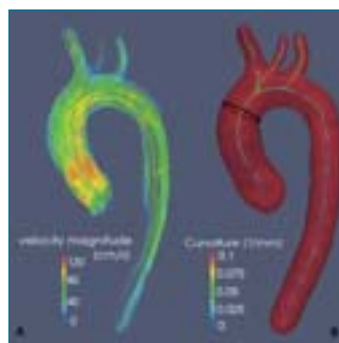
Finally, a 3D triangular surface mesh of thoracic aorta in the peak of systole was created. This 3D model, together with the velocity data, was exploited to provide a comprehensive morphometric and hemodynamic characterization of the vessel of each subject. In particular, we made quantitative measurements of blood velocity and flow, aorta area and diameter considering different planes.

Results and discussion

Qualitatively, after ADF application on PCMRI data, image noise was visibly reduced, while gradients associated to the image features were preserved. In fact, the noise characterizing the velocity images decreased after filtering and, in agreement with this, the value of divergence was reduced at least by 320%. Improvements in visualization of streamlines and secondary flow were observed for all the SENSE reduction factors applied in PCMRI acquisitions; streamlines are longer and more regular than in the not filtered data, due to the more regular 3D velocity field. In fig.1_A the streamlines calculated in systolic phase after ADF application are shown. Two main context of applications

can be found: clinics, where improved both the visualization (streamlines and pathlines) and the analysis of the flow, with the possibility of speed up the acquisition, and Computational Fluid Dynamic (CFD) simulations where the regularized velocity field can allow a quantitative direct analysis of complex patient-specific 4D flow pattern. The segmentation method had proven to be able to properly segment the thoracic aorta in subjects acquired with and without SENSE parallel imaging with results comparable to the manual contour delineated by two experts. Quantitative analysis has confirmed the good behaviour of the method: the mean distance between the contours is comparable to half of a pixel (mean distance = 1.07 mm) in the non SENSE dataset and was equal to 1.36 mm for the SENSE dataset. The 3D mesh representing the thoracic aorta, showed, in all subjects, a realistic shape, characterized by a degree of smooth comparable to a physiological vessel. Through the proposed approach, it was possible to easily and automatically calculate both morphometric and hemodynamic indices on seven planes along the vessel. In fig.1_B the 3D patient-specific vessel model is shown. These information automatically calculated can be used not

only to study the complex relationships between the geometry and hemodynamics within the aorta in pathological and in healthy subjects but also to define new and simpler indices that can be used to describe and quantify the presence and the progression of CVD. Finally, the 3D mesh of the thoracic aorta could be easily assimilated into computational fluid dynamics frameworks to get even more realistic computational hemodynamic models using the velocity values extracted in cross-sectional planes as boundary condition.



1A. blood flow visualization using streamlines of PCMRI dataset after ADF application.

1B. Patient specific Thoracic Aortic Model: 3D mesh, centerline and its curvature and a vessel contour calculated from one orthogonal plane.

INDUSTRIALIZATION AND PRE-CLINICAL TESTING OF AN OSCILLATING PERFUSION BIOREACTOR (OPB) FOR TISSUE ENGINEERING STRATEGIES

Caterina Turrisi - Relatore: **Prof.ssa Sara Mantero**

Advanced therapies and in particular tissue engineering strategies have encountered an increasing interest and a rapid evolution in the last couple of decades, due to their potential to significantly impact current therapeutic modalities by providing a virtually unlimited supply of patient-specific tissues and organs. Increasing in the number of advanced therapies undergoing both early and late-stage clinical trials as well as FDA-approved commercial products that have already entered the market strongly indicates that these therapies are emerging as a distinct healthcare sector. The translation of successful research results into the clinic however still suffers of important issues, such as cost, time, lack of ease of application and difficulty in complying with regulation. In this context the field of automated cell cultivation using highly specialized bioreactor designs and stringent bioprocess controls will be crucial for the development of biomanufacturing technologies suitable for clinical-grade production of advanced therapies. Many types of bioreactors have been designed to provide different stimuli in relation to the specific tissue to be developed. Among these, perfusion bioreactors have proven to be particularly

promising in engineering different type of tissues (bone, cartilage, heart). They are employed in tissue engineering procedures to perform specific and important functions such as cell seeding on porous scaffolds and confined medium perfusion through porous scaffolds seeded with cells. Moreover they: a) allow to overcome the typical drawbacks of manual procedures and improves efficiency of seeding processes and uniformity of cells distribution inside porous scaffolds, promoting the achievement of more uniform engineered constructs; b) improve efficiency of oxygen and metabolites transfer and catabolites removal c) allow automation and monitoring of culture medium exchange procedures; d) allow physical stimulation of cells seeded into porous scaffolds, through shear stress generation; e) reducing the use of manual actions, promote the transfer of Tissue Engineering procedures from research to clinical application, improving the traceability, reproducibility, efficiency and safety of processes (key requirements for these procedures to compete with traditional therapeutic alternatives in terms of cost, Quality Control and Good Manufacturing Practice). Despite a high number

of bioreactors already in the marketplace, a device accounting for all the requirements needed to be successfully used into a streamlined bioreactor-based advanced therapy strategy is still a lack. In this context the present study focuses on the industrialization and the pre-clinical testing of a technological platform – based on a prototype bioreactor (OPB, Oscillating Perfusion Bioreactor) – for tissue engineering purposes. Aim of the project was to develop a scalable and robust bioreactor, enabling flexible culture strategies and monitoring and control of the culture environment, taking into account serial manufacturability and quality assurance, for the cost effective and automated manufacturing of biological tissues. The study comprised two main phases: industrialization and pre-clinical testing. During the industrialization phase the prototype version of the bioreactor has been re-designed in order to comply with regulatory requirements in term of GMP practice for cell and tissue culture. In particular a scalable and robust bioreactor, enabling flexible culture strategies and monitoring of the culture environment, taking into account serial manufacturability and quality assurance, for the cost effective and automated

manufacturing of biological tissues, has been developed. Thanks to the attention paid during the design phase on the industrial design concepts and on GLP and GMP, the bioreactor proved to be easy in setting up and in managing all the functionalities, being able to receive patient-derived cells and supporting scaffold, to seed and culture them on the scaffold under perfusion condition and to deliver an engineered construct suitable for implantation. Furthermore the developed bioreactor is compact and therefore easy to be used inside a standard cell culture incubator. All parts have been designed to facilitate the procedures and to reduce the manual operations. The implementation of a sensing system able to monitor the culture parameters allows meeting traceability requirement, which is a key aspect in quality control management.



1. Industrialized OPB

A validation activity has also been carried out in order to demonstrate the correspondence with the requirements and the robustness and the safety of the device. It comprised: a) installation qualification (IQ); b) operational qualification (OQ); c) performance qualification (PQ), with the execution of sterility,

LAL and media fill tests. Pre-clinical testing has been carried out in order to validate the bioreactor and comprised:

- a. Experiments in order to verify the performances of the bioreactor in seeding and culturing several cell types. A study aiming at optimizing seeding process through Design of Experiment statistical method has been carried out. Once optimized seeding parameters using Ultrafoam scaffolds and MG63 these parameters were then used in seeding other scaffold and cell types. Optimization through DoE allowed identifying which parameters influence the seeding results. In particular we found that the flow velocity and the seeding time influence the seeding efficiency, while the seeding density influences the cell viability. As to scaffold and cell type main differences in seeding efficiency were found in relation to scaffold type and in particular to its permeability. In performing culture the bioreactor proved to be reliable and robust and was able to deliver constructs with higher cell content and viability and with a better distribution of cells throughout the scaffold thickness with respect to statically cultured ones.
- b. As final step of validation, in vivo implant and evaluation of bone grafts generated by means of the developed perfusion bioreactor have been performed in order to verify the performance of the device in carrying on a complete tissue engineering process. Ovine animal model has been chosen. Pre-

implantation analysis on grafts confirmed the effectiveness of the bioreactor in carrying out automatic and safe tissue engineering processes, delivering constructs with an osteogenic leaning. The effect of the dynamic culture conditions on the cell differentiation was investigated by studying the expression of genes involved in osteogenic differentiation and characteristic of bone tissue. The RT-PCR results showed that the perfusion is a valid stimulus in addressing cells toward the osteogenic lineage, in presence of osteogenic culture medium. Both early and late markers are more expressed in dynamically cultured constructs than in statically cultured ones for ENGIpore scaffolds.

MULTI-PERSPECTIVE INVESTIGATION OF THE EFFECTIVENESS OF ANTI-THROMBOTIC TREATMENTS IN ASSOCIATION WITH SHEAR-MEDIATED PLATELET ACTIVATION

Lorenzo Valerio - Advisors: **Prof. Alberto Redaelli, Prof. Gianfranco B. Fiore, Prof. Marvin J. Slepian**

Ventricular assist devices (VADs), the most prominent solution for treatment of heart failure (HF), are still burdened with several post-implant complications like pump failure, infections or thrombotic events. Increased shear stresses are a hallmark of flow conditions in blood recirculating devices, and patients implanted with such devices require lifelong anti-thrombotic therapies to counteract the high risk of thromboembolism. Although these agents have proven their effectiveness as biochemical inhibitors of platelet activation, their behavior under shear stress, i.e. in response to physical forces encountered when the blood passes through VADs, has been only marginally investigated.

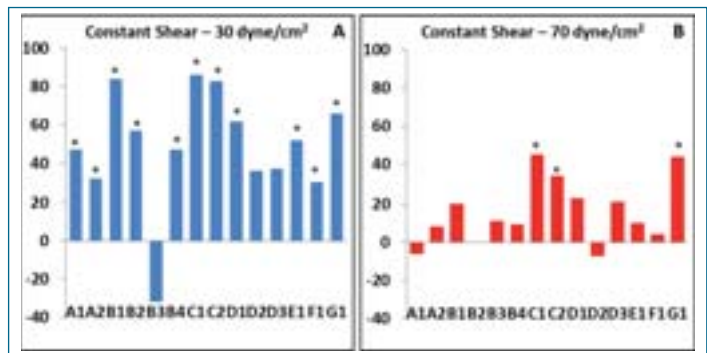
In the present dissertation a detailed investigation is performed to assess the ability of traditional and unconventional antithrombotic treatments to protect platelet from shear-mediated activation. In particular, both commonly used antithrombotic drugs and unconventional chemical agents (DMSO) are tested under constant and dynamic shear stress conditions with the aim of identifying the best mechanism of action able to inhibit shear-mediated platelet activation, thus paving the road towards a viable approach that

will be useful in assessing and developing new more effective anti-thrombotic pharmacologic agents.

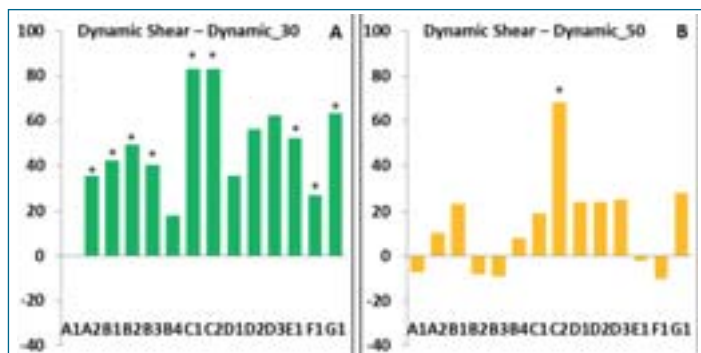
In the first part of the thesis, we aimed at investigating the effect of nowadays on the market anti-thrombotic therapies on shear-induced platelet activation after shear stress exposure via the hemodynamic shearing device (HSD), a computer controlled cone-plate viscometer able to reproduce with high fidelity the dynamic shear stress profiles encountered by blood within VADs.

We subjected gel-filtered platelets (GFP) pre-treated with drugs to different shear stress profiles, either constant or

dynamic. For what concerns the constant conditions, platelets were subjected to 30 and 70 dyne/cm² for a total time of 10 min via the HSD. On the other hand, the used dynamic shearing profiles were extracted from the stress accumulation (SA) distribution (probability density function, PDF) calculated by mean of CFD simulations within the DeBaKey VAD. The profiles corresponding to the 30th (Dynamic_30) and 50th (Dynamic_50) percentiles of the PDF were implemented in the HSD. Platelet activity state after exposure to shear stress was monitored using a specific prothrombinase assay, the PAS assay.



1. Mean % of platelet activation reduction provided by different antiplatelet agents (A1-G1) tested after 10 min exposure to 30 dyne/cm² (A) and 70 dyne/cm² (B). % reduction are intended compared to control group (* p < 0.05). Antiplatelet agents: A: aspirin alone (A1 - 25 µM, A2 - 125 µM); B: aspirin in combination with other drugs (B1- ASA 25 µM + dipyridamole 5 µM, B2 - ASA 25 µM + eptifibatide 0.25 µg/ml, B3- ASA 25 µM + pentoxifylline 100 µM, B4- ASA 25 µM + eptifibatide 0.25 µg/ml + pentoxifylline 100 µM); C: ticagrelor (C1 - ticagrelor 10 µM, C2 - ticagrelor 100 µM); D: dipyridamole (D1 - dipyridamole 5 µM, D2 - dipyridamole 10 µM, D3 - dipyridamole 25 µM); E1: eptifibatide 0.25 µg/ml; F1: pentoxifylline 100 µM; G1: cilostazol 50 µM.



2. Mean % of platelet activation reduction provided by different antiplatelet agents (A1-G1) tested after 10 min exposure to Dynamic_30 (A) and Dynamic_50 (B). % reduction are intended compared to control group. (* p < 0.05) See Fig. 1 for details regarding antiplatelet agents tested.

The antiplatelet agents investigated were Aspirin, Dipyridamole, Cilostazol, Pentoxifylline, Eptifibatide and Ticagrelor.

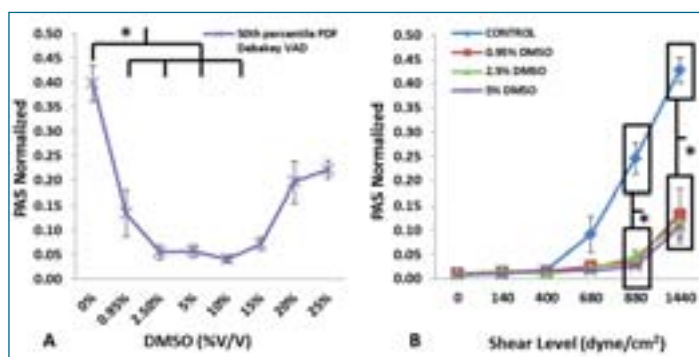
The percentage of platelet activation reduction calculated for all kinds of samples treated with drugs compared to control, are represented in Figures 1 and 2.

At 30 dyne/cm² the majority of the tested agents showed a protection effect, with a mean reduction of 55% (Figure 1-A). The same trend was found with platelets subjected to the Dynamic_30 condition (mean reduction of 50% compared to control) (Figure 2-A). On the other hand, at higher shear stress (70 dyne/cm² or Dynamic_50) only Cilostazol and Ticagrelor, respectively corresponding to the drug displayed as G1, C1 and C2, seemed to protect platelets. Results obtained suggest that the most common antiplatelet agents, which are normally used in anticoagulation management for patients treated with mechanical cardiac devices, are only partially able to protect platelets from the activation

effects of physical forces encountered by flowing through cardiac assist devices.

New mechanisms of action were also studied to overcome the limitations associated with current therapies. Dimethyl sulfoxide (DMSO) was used to modulate intactness and fluidity of the platelet membranes with the final goal of reducing shear-mediated platelet activation. Membrane integrity and its capacity to respond to external stimuli plays a key role in the mechanotransduction apparatus, which is responsible of shear-mediated platelet activation.

Results obtained after exposure of DMSO-treated platelets to different level of shear stress are represented in Figure 3. Our studies indicate that a paradigm shift is required in the development of new antiplatelet drugs for the treatment of shear mediated platelet activation. In particular, the discovery of new agents able to affect platelet membrane fluidity may reduce the need of large antithrombotic therapies, offering an effective protection to platelets when exposed to high shear stress conditions as within VADs.



3. Ability of DMSO to modulate shear-mediated human platelet activation. Pre-treatment with DMSO at specified concentrations (10 min., 37°C). Data are represented as mean ± SEM. (* p < 0.05).