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DOCTORAL PROGRAM IN BIOENGINEERING

The Doctoral Programme in Bioengineering trains graduate students through a strong interdisciplinary education on engineering, mathematics, medical and biological knowledge to develop high level engineering problem-solving abilities in life sciences inside a research group or in private or public industrial context. Students are involved in research works in fields currently ongoing at the Bioengineering Department of Politecnico di Milano which organizes the PhD track.PhD students in Bioengineering are about 20 per year, around 60 in the three year course. Research themes include modelling and analysis of physiological data, signals and systems; biomedical imaging processing and technologies; technologies and instrumentation for movement analysis, rehabilitation, ergonomics and sports; therapeutic devices and life support systems in cardiology, cardio/surgery and pneumology; design and assessment of prostheses; computer aided surgery and surgery optimization through modelling; cardiovascular fluid dynamics; molecular, cellular and tissue engineering for biomaterials and prostheses; neuro-engineering and nanobiosystems; genomic and proteomic data analysis; bioinformatics. Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the student training.

The educational offer includes ad hoc advanced courses specifically projected for the PhD. Among them, the school of the National Bioengineering Group is held every year since 1981 for one week in Bressanone (BZ). The content of the School is focused on themes of the bioengineering research and knowledge and it is organised with the support of national and international qualified teachers in the specific field coming both from academic and industrial research. The school is also a unique opportunity to put together students from different Doctoral Programs coming from the entire country. This allows exchanging ideas and experiences also representing a very useful educational event.

Some themes of the recent editions:
2006 Neuro-Robotics. Neuroscience e robotics for the development of intelligent machines
2007 Computational Genomics & Proteomics
2008 Wearable Intelligent Devices for Human Health and Protection
2009 Bioengineering for Cognitive Neurosciences
2010 Synthetic Biology
2011 Neuroinformatics
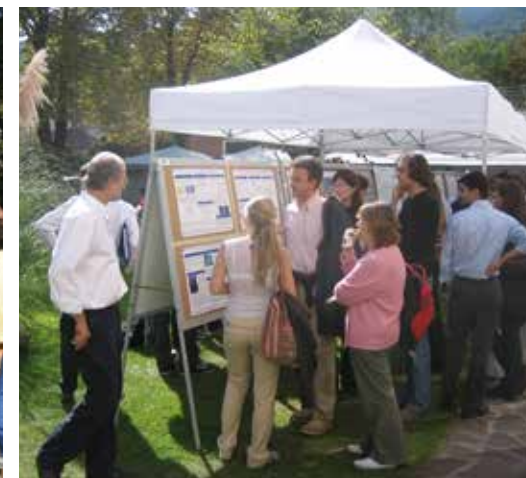
Scientific and research PhD activities receive a strong support by Laboratories located inside and outside the Department in cooperation with other research bodies and university hospitals:

· Laboratory of 2D-3D analysis and modelling of neural and sensory systems and bioelecromagnetism
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· Laboratory of biocompatibility and cell culture -BioCell
· Laboratory of Biological Structure Mechanics – LABS
· Laboratory of Computational Biomechanics
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The interest toward the activities of the Ph.D in Bioengineering is demonstrated also by the external financing of 3 years PhD Fellowships. Some recent supporters, besides the Bioengineering Department, of our PhD are:

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In 2010 and in 2011 new PhD positions as Executive PhD's have been created. They consist of a special PhD path organized in 4 years and dedicated to PhD candidates that already work in a company/society.
The Bioengineering PhD opened 3 positions in 2010 (Fraunhofer Institute, Erlangen, Germany; Istituti Ortopedici Rizzoli, Bologna; SKE S.r.l. Milano) and in 2011 (Medtronic SpA, IRCCS Besta, Milano).

AUTONOMIC REGULATION OF THE CARDIO-RESPIRATORY INTERACTIONS DURING SLEEP - INTERPRETATION THROUGH A CARDIORESPIRATORY SYSTEM MODEL

Ramona Cabiddu - Supervisor: **Cerutti Sergio**

Cardiovascular rhythmicity is modulated by the autonomic sympathetic and vagal influences; thus, by analyzing the Heart Rate Variability (HRV) signal, it is possible to investigate the autonomic cardiovascular control. Time domain and frequency domain HRV analysis has proven to be a powerful tool in the detection of autonomic dysfunctions in pathological conditions, including pathologies affecting the cardiovascular system. Cardiovascular disease is the leading cause of death in developed countries and, also because of this, researchers have focused in recent times on the development of markers for the diagnosis and prognosis of cardiac pathologies and for the cardiovascular risk stratification. Recently, a growing interest has been manifested towards the application of non-linear methodologies of analysis to the HRV signal, which might provide a more insightful description of the HRV dynamics.

In recent years, great interest has been manifested on the cardiovascular control during sleep, also given the evidence that many sleep disorders have been proved to be associated with cardiovascular disorders. Sleep is physiologically characterized by changes in the autonomic regulation of the cardiovascular activity. 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 vagal and sympathetic influences, during rapid eye movement (REM) sleep.

Like cardiac activity, respiration undergoes important modifications during sleep as well, physiologically becoming more regular during deep sleep and more frequent during REM sleep. The association between cardiac and respiratory rhythms has been widely recognized and evidence exists that it can be impaired by disease affecting the respiratory system. Also because of this, in recent years the study of the cardiorespiratory coupling has elicited great interest among sleep researchers.

The final dissertation was articulated around two major aims. The first part was devoted to present the results of the clinical studies we conducted to investigate the autonomic cardiac regulation and the cardiopulmonary coupling during sleep on healthy and pathological subjects.

The first four studies consisted in frequency domain investigations of HRV and cardiorespiratory coupling during sleep in a population of healthy subjects and in different pathological populations.

The first study, published on *Frontiers in Physiology* under the title "Modulation of the sympatho-vagal balance during sleep - Frequency domain study of heart rate variability and respiration", aimed at assessing autonomic cardiac regulation and cardiopulmonary coupling during sleep in healthy subjects, using spectral analysis of the HRV and respiration signals. In line with previous evidence, results attested a sympatho-vagal balance shift towards vagal modulation during NREM sleep and towards sympathetic modulation during REM sleep. A higher cardiorespiratory synchronization was observed during deep sleep.

The second study was performed with the aim to investigate the autonomic control of cardiac and respiratory activities in difficult-to-control asthmatic patients during sleep. Our findings suggest that an impairment of autonomic regulation characterizes this pathology, which leads the sympathetic control to be predominant over vagal control, possibly contributing to the poor quality of sleep observed in these patients. Sympatho-vagal alterations may be related to the severity of the disease and may be useful for better clinical management of patients.

The paper from which the third study was extracted, titled

"Heart Rate Variability and Cardio-Respiratory Coupling during sleep in Patients prior to Bariatric Surgery", has been recently published on *Obesity Surgery*. The objective was to determine the relationship among severity of obesity, autonomic cardiac regulation and cardiorespiratory coupling in obese patients during sleep. Results attested reduced HRV and respiration regularity in these patients. A relationship among autonomic impairment and severity of obesity was found.

The fourth study was performed with the objective to investigate the autonomic cardiorespiratory regulation during sleep in elderly patients affected by Obstructive Sleep Apnea (OSA) and to compare these results with those obtained from younger OSA patients. OSA elderly patients presented more pronounced alterations in cardiac autonomic modulation and cardiorespiratory coupling.

The last two studies consisted in the application of non-linear methodologies to investigate HRV complexity during sleep in two pathological populations. The first of them aimed at investigating if and how a set of HRV derived parameters change in heart failure patients with respect to normal subjects. Our observations support the hypothesis of a modified cardiovascular autonomic modulation in heart failure patients. The possibility of employing parameters derived from HRV signals recorded during sleep to discriminate normal subjects and heart failure patients is supported by our results.

The second of them was

performed with the aim to analyze HRV complexity during wakefulness and sleep in healthy and obese subjects. Our findings support a central role of excessive adiposity in influencing HRV during sleep and show that HRV complexity is reduced in obesity.

The presented studies serve the purpose of providing a better knowledge of the pathophysiological state in the investigated conditions, a deep understanding of which is fundamental to assist experts in the diagnosis and management of pathologies. In this field, great importance is attributed to the modeling of physiological systems with the aim to realistically simulate the system behavior in physiological and pathological conditions. The PNEUMA model was developed by the researchers of the University of Southern California to meet this necessity.

The final part of the work was dedicated to give an interpretations of the experimental results obtained, using the PNEUMA model. A set of simulations were performed with the aim to reproduce the status of the autonomic regulation system observed in our previous studies on healthy subjects and different categories of patients. The objective was to interpret our experimental results and to give a more complete characterization of the cardiorespiratory status in each of the analyzed pathological populations.

For all analyzed conditions deviations from the physiological behavior of the cardiorespiratory autonomic modulation and of the cardiorespiratory coupling were observed, and analogous

alterations in the estimated cardiorespiratory parameters were observed.

Our results might represent an interesting starting point to improve the therapeutic strategies currently available for the management of the investigated pathologies. The cardiorespiratory alterations observed in the different pathological populations suggest that the treatment and rehabilitation of the respiratory function are of great importance not only for the restoration of the respiratory function itself, but also for the preservation of autonomic and cardiac functionality.

Available knowledge about the relationships between sleep-disordered breathing and cardiovascular disease remains incomplete. The complex dynamics of the physiological events underlying respiratory instability during sleep make it difficult to distinguish cause from effect. A mathematical modeling approach could make it possible to separate the influences of the interacting physiological mechanisms and to reach a better understanding of how each of them contributes to the complex dynamics of the cardiorespiratory control in different conditions.

SILK FIBROIN SINGLE LAYER AND TWO-LAYER TUBULAR SCAFFOLDS FOR SMALL DIAMETER BLOOD VESSEL REGENERATION

Valentina Ilaria Maria Catto - Supervisor: **Silvia Fare', Giuliano Freddi**

Bypass surgeries are commonly performed to allow the peripheral or coronary revascularization. To date, the clinical approach for the replacement of small diameter blood vessels (inner diameter (ID) < 6 mm) is the use of autografts, despite the fact that they may not be the optimal solution. Tissue engineering has become a promising approach for vascular regeneration. Although vascular tissue engineering has reached promising results in clinical trials, tissue-engineered vascular grafts (TEVGs) exhibit some drawbacks, such as the regeneration of nonfunctional endothelium, a mismatch between the mechanical proprieties of grafts and natural blood vessels, long manufacturing times.
Among available materials for fabrication of TEVGs, natural polymers exhibit good biological performances but usually lack the mechanical properties necessary for *in vivo* implantation. In contrast, silk fibroin (SF) excels for its peculiar mechanical properties and high biocompatibility.
The present PhD thesis aims to design, fabricate and characterize innovative scaffolds based on SF for the regeneration of small diameter blood vessels able to overcome the limits of the autografts.
Nanostructured electrospun SF (ES-SF) tubular scaffolds with
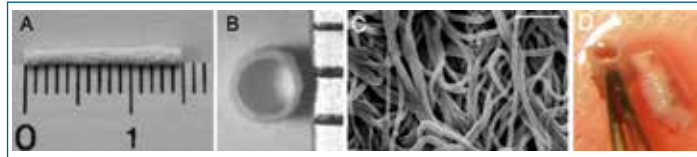
1. Macrographs of the length (A) and the diameter (B) of ES-SF tubes; SEM image of ES-SF tubes (C, scale bar = 5 µm); macroscopic image of retrieved graft after 7 days (D).

1.5 mm ID were successfully fabricated for the first time (Fig. 1A, B); in fact, 1.5 mm ES-SF tubes are novel SF scaffolds, not yet reported in the literature. Scanning electronic microscope (SEM) analysis showed a homogeneous and random fiber distribution in the nanometric range (Fig. 1C). ES-SF morphology allowed for the *in vitro* adhesion and growth of primary porcine smooth muscle cells (SMCs). Specifically, SMCs seeded on external surface of the ES-SF tubular scaffold were able to migrate inside tubes, reaching the lumen and demonstrating an appropriate scaffold porosity for cell migration. The results obtained by the *in vitro* characterization appear promising for the investigated final application. ES-SF tubes were mechanically characterized, exhibiting appropriate mechanical performance for the specific application. In fact, ES-SF tubes showed higher ultimate tensile strength (UTS) in circumferential direction than anterior

descending human coronary arteries and higher strain at break (ε_b) in both directions than human saphenous veins, the gold standard for arterial bypass grafts. Furthermore, the suture retention strength (SRS) was similar to human grafts and the burst pressure (BP), calculated by rearranging the Laplace's law, was higher than the upper physiological pressures, but lower than native human saphenous veins.
ES-SF tubes were evaluated *in vivo* in a rat model in the short period. Acellular ES-SF tubes were implanted in the abdominal aorta of Lewis rats by end-to-end anastomosis. After 7 days, rats exhibited no signs of acute thrombosis and occlusion and the graft lumen showed the absence of aneurismal dilatation and apparent intimal hyperplasia (Fig. 1D). ES-SF tubes allowed the *in vivo* regeneration of a vessel-like structure similar to the native blood vessels, specifically inducing the elastin regeneration that only few TEVGs described in literature
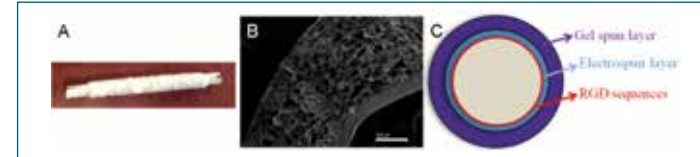
2. Macrographs of the two-layer SF tube length (A); SEM image of two-layer SF tube (C, scale bar = 200 µm); (C) sketch of the cross-section of two-layer SF tube.

are able to promote. The *in vivo* favorable interaction between host cells and ES-SF tubes may be due to the combination of SF peculiar properties and the ES technique that allows for the fabrication of porous structures with nanofibers and high surface to volume ratio. These preliminary results showed that the ES-SF tubes would be promising off-the-shelf scaffolds for the regeneration of small diameter blood vessels.
To better mimic the native structure of blood vessels, novel two-layer SF scaffolds were developed by the innovative combination of two techniques, the ES and the gel spinning (GS) (Fig. 2A, B). In particular, two-layer SF tubular scaffolds consisted of an ES-SF tube coated with a GS-SF layer, specifically the ES layer acted as the tunica intima and the GS layer mimicked the tunica media. To enhance the endothelial cell adhesion and proliferation, the lumen of the two-layer SF tubes was functionalized with the RGD sequences

(Fig. 2C) by an innovative combination of carbodiimide and diazonium coupling to enhance the efficiency of the RGD functionalization. Two-layer SF tubes were mechanically characterized demonstrating similar or higher mechanical properties than native blood vessels. Specifically, two-layer SF tubes showed higher UTS in circumferential direction than anterior descending human coronary arteries and higher ε_b than human saphenous veins in both directions. Furthermore, the SRS of two-layer SF tubes is in the range of that of human grafts and the two-layer SF tubes demonstrated a BP similar to native human saphenous veins. Two-layer SF tubes were seeded in the lumen and on the outer surface with primary human aortic endothelial cells and primary human aortic smooth muscle cells, respectively. Cell-seeded scaffolds were *in vitro* cultured for 7 days in a perfusion bioreactor. The results confirmed the biocompatibility of the two-layer SF tubes and their ability to

support adhesion and growth of primary human cells.
The developed single-layer ES-SF and two-layer ES-SF/GS-SF tubes exhibited promising performance for small diameter blood vessel regeneration, in terms of morphological, mechanical and biological behavior.

NUMERICAL MODELING OF HEMODYNAMICS IN STENTED CORONARY ARTERIES

Claudio Chiastra - Supervisor: **Prof. Francesco Migliavacca**

Coronary heart disease (CHD) is one of the major causes of death and premature disability in developed societies. It is caused by atherosclerotic lesions that reduce arterial lumen size through plaque formation and arterial thickening, decreasing blood flow to the heart and frequently leading to severe complications like myocardial infarction or angina pectoris. PCI, which consists in balloon angioplasty usually followed by stenting, is the most commonly performed procedure for the treatment of CHD. This procedure is still associated to serious clinical complications such as in-stent restenosis (ISR), which is the reduction of the lumen size as a result of neointimal hyperplasia (NH), an excessive growth of tissue inside the stented vessel. The phenomenon of ISR has been partially attenuated by the introduction in 2004 of drug eluting stents, which are able to release antiproliferative drugs with programmed pharmacokinetics into the arterial wall. However, restenosis rate remains higher than 10% when complex lesions (e.g. bifurcation lesions) are treated. The mechanisms and the causes of ISR are not fully understood. In addition to vascular injury caused by device implantation and foreign-body reactions, hemodynamic alterations
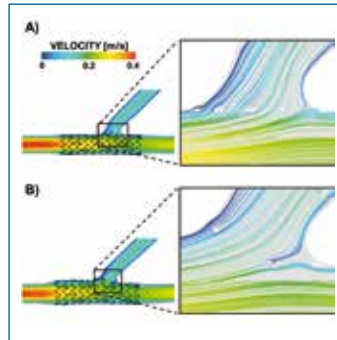
1. Velocity contours and streamlines at the average flow rate in the transversal plane after (a) the main branch stenting and (b) the FKB.

induced by the stent presence can be associated with NH. Therefore, the study of the fluid dynamics of stented coronary arteries is of extreme importance for a better comprehension of the mechanisms involved in ISR. In this context, the present thesis is focused on the numerical modeling of hemodynamics in stented coronary artery geometries. Indeed, computational fluid dynamics (CFD) allows the investigation of local hemodynamics at a level of detail not always accessible with experimental techniques, calculating fluid flow variables (e.g. wall shear stress – WSS) that can be used as indicators to predict sites where NH is excessive.
The thesis is characterized by three main topics:

· *the study of the effect of wall compliance of stented coronary artery models on hemodynamic quantities*. The results of fluid-structure interaction (FSI) models of a straight stented coronary artery were compared to the corresponding rigid-wall models. Two different materials were considered for the stents, i.e. cobalt-chromium and poly-L-lactide. Similar results were found in terms of time-averaged and instantaneous WSS between compliant and rigid-wall cases. These results indicate that, for idealized models of a stented coronary artery, rigid-wall assumption for fluid dynamic simulations is adequate when the aim of the work is the study of near-wall quantities;

· *the comparison, from the fluid dynamic perspective, of different stenting procedures for the treatment of bifurcation lesions*. Rigid-wall fluid dynamic simulations were performed on idealized stented coronary bifurcation models. A hybrid meshing strategy, which uses both tetrahedral and hexahedral elements, was applied for the creation of the meshes in order to reduce the computational costs.
Firstly, final kissing balloon (FKB) inflation within provisional side branch (PSB) approach, which nowadays is the preferred

bifurcation stenting strategy, was investigated. Results of CFD analyses highlighted the advantages of FKB in terms of flow pattern and access to the side branch (SB) (Fig. 1), but also its drawbacks in terms of overexpansion of the proximal main branch (MB) (wider region with low and oscillating WSS). Secondly, the different hemodynamic scenarios provoked by PSB performed with a proximal or a distal access to the SB were compared. WSS distribution, velocity and helicity fields were superior for the distal access, giving a quantitative support to the clinical experience that suggests to perform the distal access instead of the proximal one within PSB approach.
Lastly, the double stenting culotte technique was studied, comparing the behavior of a conventional stent with the dedicated stent Tryton (Tryton Medical Inc.). The use of this dedicated device reduced the amount of double metallic layers in the proximal MB, which represents a critical point of the culotte technique. Moreover, fluid dynamic results were found improved in the Tryton-based model. In fact, the particular design of Tryton markedly decreased the areas with low WSS and high relative residence time (RRT);
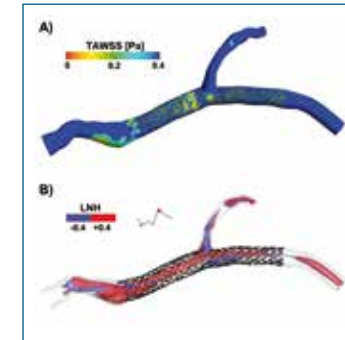
2. a) Contours of time-averaged WSS (TAWSS) for one imaged-based stented coronary artery model. b) Isosurfaces of local normalized helicity (LNH) at peak flow rate. Positive and negative values indicate counter-rotating flow structures.

· *the study of the hemodynamics of image-based stented coronaries*. Two cases of pathologic left anterior descending coronary arteries with their bifurcations reconstructed from computed tomography angiography and conventional angiography were studied, calculating both near-wall and bulk flow quantities. Results of WSS and RRT showed that the regions prone to the risk of restenosis are located next to stent struts, to the bifurcations and to the stent overlapping zone (Fig. 2a). Looking at the bulk flow, helical flow structures were generated by the shape of the vessel upstream from the stented segment and by the bifurcation (Fig. 2b). Helical

recirculating microstructures were also visible downstream of the struts.

Moreover, reconstruction methods of *in vitro* and *in vivo* stented coronary models for CFD simulations were developed starting from optical coherence tomography (OCT) images. OCT is a promising tool to reconstruct 3D geometries, due to its high spatial resolution and the possibility to detect both the stent and the vessel. Although the developed methodology is preliminary, it represents a first step towards the semi-automatic creation of image-based stented coronary models for CFD simulations.

In conclusion, the main achievements of this thesis are the following: (1) the implementation of a FSI model of a stented coronary artery; (2) the development of a hybrid meshing strategy for reducing computational costs of CFD simulations; (3) the fluid dynamic assessment of different stenting procedures for the treatment of coronary bifurcations; (4) the hemodynamic analysis of image-based models of stented coronary models which replicate a real stenting procedure; (5) the development of reconstruction methods of stented coronary models from OCT images.

HIGH LEVEL CONTROL OF ROBOT BEHAVIOR IN NEUROSURGERY

Mirko Daniele Comparetti - Supervisors: **Prof. Giancarlo Ferrigno, Elena De Momi**

Surgery is a very complex task that is performed by humans in tough conditions: stress and, eventually, reduced working space, can make the task more and more complex.

In order to assist the surgeon during the intervention, engineering research in the converging measurement, computer graphics and bio-medical fields led to the development of Computer Aided Surgery (CAS) techniques. This is a set of methods that can assist the surgeon starting from the pre-operative phase to the intra-operative phase, providing tools to check, during the intervention, the correctness of the procedure through visual feedback on the medical images or augmented reality. This can be done using the diagnostic images, performed prior to the intervention on which the surgeon plans the surgical procedure, after a proper registration with the intra-operative reality.

CAS procedures were introduced at the beginning of the 1990's, but the basic concepts here used were already introduced in the surgical practice with different devices. One of these was the stereotaxic frame, introduced in the beginning of 1900; it is a device that is fixed on the patient's head and allows the insertion of straight instruments (i.e. electrodes or

probes for biopsies and localize drug delivery) using a Cartesian Coordinate Frame defined by the frame itself, after properly mounting and aligning it with anatomical landmarks.

In the last decades, robotics increased its role in surgery: Computer and Robot Assisted Surgery is an extension of the concept of CAS in which there is the interaction with the patient through the use of a robot arm. The use of robots in surgery is a valid tool to aid the action of the surgeon, providing an active support along with the increased number of information provided by CAS. Anyway, the robotic device cannot substitute completely the surgeon, but it can be used exploiting its features in order to increase the possibility of the surgery and alleviate difficulties, also providing the surgeon with information that can be used to improve the performances of the treatment. The robotic assistant have to act according to the needs of the surgery and the surgeon, as an intelligent transparent operator which ensures an higher accuracy and a better performance, with respect to traditional techniques, by reducing the fatigue to the human operator and providing a reliable way to verify and improve the accuracy of the procedure.

In this thesis, the aspects of high

level control of a robotic device for neurosurgical intervention was studied and an architecture (presented in figure) to manage a robotic system during the execution of the workflow of the intervention was developed in order to change the parameters and control modes in a semi-automatic way, according to the current situation in the OR, the step of the intervention and the surgeon's needs. In detail, the work was focused in the definition of a set of Finite State Machines (FSMs) that can manage the transitions between two steps by properly enabling/disabling the control modes and parameters without causing unpredictable movements and glitches of the robot, which is close to the patient. For doing so, a simplified surgical procedure was designed in which the robot exploits all the possible control modes foreseen in the different step of the procedure: 1) autonomous movement, 2) cooperative control, and 3) tele-operated control; the user, though a proper User Interface, makes the request to switch to the following step and the control architecture triggers the proper smooth transition on the FSM, towards the desired state. To test the architecture, this procedure was repeated 19 times and the data from both the robot encoders and an external tracker

were recorded and the positions before, during and after the event were compared among each other to identify differences related to an undesired movement of the robot arm; the study was also performed on the first three derivatives of those signals.

In this thesis, also controllers to move, with a great accuracy, a tool carried by a robot towards a pre-calculated target pose in space were developed and tested; this procedure, called targeting, was studied with a target 1) that doesn't move in space, and 2) that can change its pose in time.

In case of a static target, the developed algorithm uses an external localizer to measure the accuracy of the position and, based on that, to iteratively correct the pose of the tool until the accuracy requirements are satisfied; studies on the final accuracy and convergence performances of the algorithm were carried out to verify that the algorithm satisfies the requirements of the intended surgical application. The test showed that the algorithm ensures an accuracy level that satisfies the neurosurgical requirement of 1mm maximum error on the target inside the brain within a limited number of iterations; the convergence study identified the limits on the possible perturbations of the

1. The robotic architecture: the robot (1) can be controlled through a master device (2) to perform an operation using a surgical tool (3)

transformation matrices involved in the complete kinematic chain, proving the robustness of the algorithm and its applicability with the noise ranges provided by the commercially available measurement systems and robotic arms.

An algorithm to follow a moveable target was implemented in the developed architecture using an external localization system to track the position of the target and the current pose of the tool. At each time frame, the new pose for the robot that brings the tool on the target is calculated and then the trajectory towards the target itself is sampled in the robot controller internal cycle rate using a trapezoidal velocity profile that constraints the accelerations and maximum velocities to the ones allowed by the robotic arm. In order to measure the performances

of the algorithm, tests were performed by defining the trajectory of the target 1) on a circle as a function of frequency and diameter, and 2) on a parabolic movement as a function of the acceleration and velocity on the trajectory.

All the developed algorithms were tested in the scope of the EU funded projects for brain surgery ROBOCAST (FP7-ICT-2007-215190) and ACTIVE (FP7-ICT-2009-6-270460), aimed at developing integrated solutions to assist the surgeons during the intervention. Those activities address the creation of the OR of the future, in which the clinical staff, sensors and the robotic assistants will share the stage with a context-driven surgical workflow that adapts the behavior of the devices without requiring a massive intervention of the human operator.

DYNAMIC CARDIOVASCULAR CONTROL OF ARTERIAL BLOOD PRESSURE AND HEART RATE IN RESPONSE TO CENTRAL VOLUME VARIATIONS AND ANESTHESIA

Guadalupe Dorantes Mendez - Supervisors: **Manuela Ferrario**

The arterial and cardiopulmonary baroreflexes are important mechanisms for short-term blood pressure regulation, and there is evidence about the clinical relevance of the analysis of these mechanisms. Maintenance of arterial blood pressure (ABP) to prevent hypotension and preservation of organ perfusion are the main challenges faced by the clinicians during hemodynamic monitoring in major surgery as well as in the intensive care unit. In this context, the aim of this thesis is to assess arterial and cardiopulmonary baroreflexes during perioperative maneuvers through mathematical models, in order to provide quantitative indices that may contribute to the characterization of hemodynamic status of patients and give additional information that could help, for instance, to support the decision making process of anesthesiologists, constantly faced with the challenge of identifying the optimal strategy to stabilize volumes and pressures during surgery.

The maneuvers that were explored in this thesis were oriented to study alterations due to anesthesia and variations in central volume during major surgery. In addition, baroreflex responses to a lower body negative pressure (LBNP) procedure before and after long duration bed rest were studied. LBNP represents a physiological model of hemorrhage, i.e. a decrease in venous return, whereas long duration bed rest is a model of cardiovascular deconditioning.

The novel contribution of this thesis lies therefore on the analysis approaches used to assess arterial and cardiopulmonary baroreflexes from non invasive or minimally invasive recordings and in the application to different experimental conditions.

Arterial baroreflex control during anesthesia induction
In this study, the causal interactions between heart rate (HR) and ABP in patients undergoing general anesthesia were quantified. The analysis of baroreflex sensitivity (BRS) through a mathematically rigorous procedure in the perioperative period could result in the availability of additional information to guide anesthesia in uncontrolled hypertensive patients, which are prone to a higher rate of hypotension events occurring during sedation.

Non hypertensive (NH) and chronic hypertensive (CH) patients undergoing major surgery were enrolled in the study. A Granger causality test was performed to verify the causal relationship between RR and systolic blood pressure (SBP), and four different mathematical methods were used to estimate the BRS. Three different surgical epochs were considered: awake, post-induction and post-intubation. A comparison of BRS trends in CH patients with respect to NH patients was performed as well.

In NH patients, propofol administration caused a decrease in ABP, due to its vasodilatory effects, and a reduction of BRS, while HR remained unaltered with respect to baseline values before induction. A larger decrease in ABP was observed in CH patients when compared to NH patients, whereas HR remained unaltered and BRS was found to be lower than in the NH group at baseline.

Arterial and cardiopulmonary baroreflex control on heart rate
In this second study, the role of arterial and cardiopulmonary baroreflex control on HR was quantified, in particular the sympathetic mediated and respiratory sinus arrhythmia mediated heart rate variability (HRV) responses to mild, rapid onset and short duration LBNP cycles, and secondly the possibility of "reverse" Bainbridge reflex by black box modeling of HRV was investigated.

In order to explain short term control mechanisms of HR and ABP, a previous mathematical model of hemodynamic variability for the description of arterial control of circulation by neural and non neural regulatory mechanisms was improved by including the relationship between central venous pressure (CVP) and RR.

The data analyzed in this study are a subset of data collected during the Women's International Space Simulation for Exploration (WISE-2005). The subjects underwent LBNP maneuver with increasing levels (from 0 up to -30 mmHg). The experiment was completed once before entry into bed rest and then repeated again on day 50 of head down bed rest (HDBR). CVP was progressively decreased with increasing LBNP intensities in both conditions (pre-HDBR and during HDBR), whereas HR significantly increased only during HDBR at high LBNP intensities, as expected.

The analysis of the impulse response of the transfer function between CVP and RR showed that the "Reverse" Bainbridge effect was elicited during mild LBNP cycles, but its limited relevance tends to disappear in the presence of cardiovascular deconditioning due to prolonged bed rest.

These results show how the rapid onset of mild LBNP does involve the cardiopulmonary baroreflex in mediating the regulation of vascular resistance, and also affects HR according to a "reverse" Bainbridge mechanism; however, this small contribution tends to become even smaller in simulated weightlessness conditions. Finally, a decrease in low frequency (LF) component of BRS gain with increasing levels of LBNP was found before and on day 50 of HDBR, suggesting a progressive impairment of arterial baroreflex with high levels of LBNP, which is more relevant with the combined effect of bed rest.

Cardiopulmonary baroreflex control of afterload and heart contractility
In the third study, an analysis was carried out to disentangle the contribution of cardiopulmonary baroreflex control of afterload and heart contractility in two different protocols with different signals recordings and settings in order to study the effects of central volume variations.

Two identification models were applied for the prediction and for the spectral decomposition of beat-by-beat fluctuations of stroke volume (SV) and pulse pressure (PP), as an extension of a previously proposed model. PP signal was used as a surrogate of SV.

In the first setting of volume unloading, data from subjects that participated in the LBNP experiment before and during HDBR were analyzed. Estimated gain of cardiopulmonary baroreflex control of ventricular contractility decreased as was expected by a reduction in venous return, in both conditions (before and in HDBR), but only in some subjects, with no significant differences.

For the study of the hemodynamic response to an increase in venous return, i.e. volume loading, fluid infusion maneuver was analyzed in patients undergoing major surgeries.

A significant decrease in the mean value of RR interval after fluid infusion revealed a possible Bainbridge reflex, i.e. hypervolemia-induced tachycardia; however, no significant changes were found in the frequency domain to have conclusive findings.

The increase of gain of cardiopulmonary baroreflex control of ventricular contractility, hinted that the cardiopulmonary baroreflex enhanced ventricular contractility to improve cardiac performance when the circulating volume was increased, but this trend was observed only in some patients. The significant increase in the contribution of CVP to PP variability prediction after fluid infusion suggests that the role of cardiopulmonary baroreflex control on ventricular contractility increases with fluid infusion maneuver, whereas the role of afterload modulation of cardiac ejection decreases.

Conclusion
The results illustrated in this thesis showed that the assessment of arterial and cardiopulmonary baroreflexes may provide useful information that could be used as a powerful tool in hemodynamic monitoring of patients.

Quantification of contribution of the role of baroreflexes could provide additional information to interpret variations of central volumes under stress conditions such as anesthesia and clinical maneuvers, and could aid in the administration of the proper therapy to ensure hemodynamic stability and to prevent unexpected and potentially harmful blood pressure drops. However, future studies and clinical protocols are needed in order to standardize and validate these results in a larger population.

IMAGE-GUIDED MANAGEMENT OF UNCERTAINTIES IN SCANNED PARTICLE THERAPY

Giovanni Fattori - Supervisor: **Prof. Guido Baroni**

In modern medicine, ionizing radiation represents an important option for selective tumor cells sterilization. The use of particles for external beam radiotherapy is a recent breakthrough technological achievement, potentially increasing the tumor control ratio by coupling the high-precision delivery with dose-escalated protocols. However, uncertainties in alignment procedures and organ motion due to patient physiology undermine the correspondence of planned and delivered dose distributions, likely resulting in poor treatment outcomes. This aspect is emphasized in particle treatments, where the high sensitivity to density variations along the beam path would result in severe dose inhomogeneity. The effective spread of particle therapy treatments is driven by the technological advances in computer-assisted therapy that are required to manage the intrinsic sensitivity to targeting uncertainties.

This work is a technological contribution towards the development of advanced procedures for image-guidance in particle therapy, aiming at enhanced setup control capabilities in the treatment of static and moving tumors.

Advanced setup control in radiotherapy

1. Custom robotic imaging system installed at CNAO

The envisaged strategy for patient setup and monitoring merges anatomical information coming from in-room X-ray imaging with continuous optical monitoring of surface surrogates, acting as redundant non-invasive geometry check during the whole treatment. The imaging part consists of a six axes articulated arm mounted on the room floor equipped with a custom C-arm tool, integrating a diagnostic kV X-ray source and an amorphous-silicon flat panel detector (Figure 1). The image-based treatment geometry verification is based on dedicated in-house developed software featuring 2D/3D registration from double planar kV-images or 3D/3D alignment by acquiring in-room volumetric cone beam CT.

The optical tracking systems (OTS) adopted in this project

are commercial off-the-shelf solutions designed for infrared motion capture in human gait analysis. The bundle setup inclusive of multiple tele-video cameras (TVC) and software tools for 3D reconstruction of spherical markers in free motion was integrated with a dedicated application for frameless stereotactic setup verification in radiotherapy based on surface fiducial markers.

Despite the adoption of state-of-art IGRT technologies and procedures aiming at the maximal accuracy, the clinical outcome is affected by uncertainties inherent to the treatment process. The actual technology installation at the Centro Nazionale di Adroterapia Oncologica (CNAO) in Pavia reported millimeter scale residual setup errors in a number of treatment fractions of stationary tumors. A critical review of the observed performance was provided, assessing the dose effect of such uncertainties in the treatment of head chordoma with carbon ion beams. Observed geometrical mismatches were implemented in the patient imaging dataset and considered for simulated treatment delivery (Figure 2). This study allowed us to move from merely geometrical setup errors quantification to the assessment of biological dose effects of such errors thus
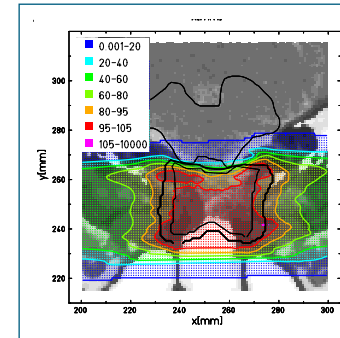
2. Simulation of carbon ion treatment of head chordoma in presence of uncertainties.

providing an insight about the treatment quality at the current stage of technology for patient setup verification.

Treatment of intra-fractionally moving targets

Despite the noteworthy research efforts to handle motion-induced uncertainties in treatment planning and delivery, the effective clinical treatment of moving lesions with active beam scanning is still a challenge. The main reason is the need to deal with deviations in the motion patterns at the treatment time with respect to the treatment planning condition, as resulting from the patient specific inter- and intra-fraction variability. The real-time integration of information coming from different motion monitoring and imaging systems in the treatment room is a necessary requirement for
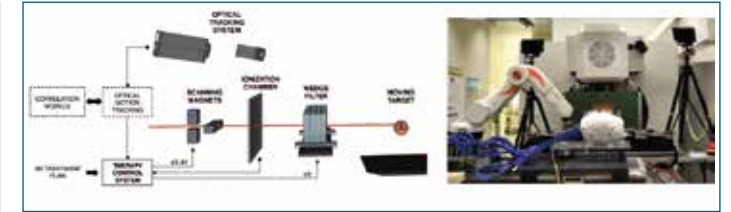
3. Concept of the integrated framework for optical driven beam tracking and installation at GSI Helmholtzzentrum für Schwerionenforschung during the experimental session with breathing phantom.

the implementation of any motion-correlated or motion-compensated irradiation strategy. To this end, algorithms for breathing motion analysis were included in the OTS software, extending its functionality to the delivery of 4D treatment plans. The proof of concept was experimentally demonstrated relying on breathing phantoms mimicking the correlated motion of a lung lesion and patient thorax (Figure 3). Two competing motion mitigation strategies currently under investigation for scanned ion therapy were implemented, i.e. gating and beam tracking. Few percent dose discrepancy between motion compensated and static irradiation were measured providing film and ionization chambers measurements.

Conclusion

The design and experimental verification activities of a comprehensive framework for image guidance in scanned

ion beam therapy is reported. The described technologies have a high impact on clinical activities in particle radiotherapy treatments at CNAO. At this institution the system is installed and daily used for automated setup verification and management of geometrical uncertainties. Overall, the innovations proposed in this work can improve the quality of care for oncological patients undergoing advanced radiotherapy treatments, ensuring high standard in setup control and verification.

BRAIN FUNCTIONAL CONNECTIVITY IN RESTING STATE: METHODS FOR NETWORKS IDENTIFICATION AND NOISE SEPARATION IN HEALTHY SUBJECTS AND ALZHEIMER'S DISEASE

Ludovica Griffanti - Supervisor: **Baselli Giuseppe**

Background and aim - Resting-state functional magnetic resonance imaging (RS-fMRI) is a widespread and powerful technique for investigating the functional connectivity (FC) of the human brain. In RS-fMRI studies, subjects are asked to rest quietly while brain images are acquired. The idea which stands behind this approach is that the brain regions similarly modulated by stimuli or tasks, rather than being idle during rest, display instead vigorous and persistent functional activity mainly detected as spontaneous though coherent low-frequency BOLD signal fluctuations. The similarity between the timeseries in different voxels can be estimated, thus providing measures of functional connectivity (FC). In this way FC is suggested to describe the relationship between the neuronal activation patterns of anatomically separated brain regions, reflecting the level of functional communication between them. With this technique it has been observed that, at rest, the brain is organized into Resting State Networks (RSNs) that can be associated with specific functions, and that can be altered in pathological conditions. Although several analysis methods are currently used for the analysis of RS-fMRI data, a common problem is

the separation of noise from the neural-related signal of the RSNs, due to the absence of a model for neural activity. Hence, effective methods for the correct identification and removal of the artefacts from the data are needed to obtain reliable FC analyses. This is particularly important in Alzheimer's disease (AD), as the decreased functional connectivity of the default mode network (DMN), quantified on RS-fMRI data, is becoming a possible new biomarker for this pathology. Therefore an early diagnosis and a detailed characterization of this alteration are crucial.
The aim of this study was to optimize and validate objective methods for the investigation of the RSNs based on RS-fMRI, in healthy subjects and patients with AD. In particular, once quantified the amount of FC estimation errors in seed-based FC analysis (one of the most common FC analysis techniques), the problem of artefact removal from the raw data was focused, in order to optimize any subsequent FC analysis. An automated denoising method (FMRIB's ICA-based X-noisefier - FIX), was developed in collaboration with the FMRIB (Functional Magnetic Resonance Imaging of the Brain) Centre (University of Oxford, UK), and was tested on different datasets (healthy controls

and AD patients), acquisition sequences (standard EPI and multi-band accelerated EPI), and group ICA model orders (low- and high-dimensional group ICA) for spatial, temporal, and network analyses. Finally, through the combination of an effective cleaning procedure and high-dimensional RSNs analysis a better localisation and quantification of FC alterations in AD was aimed at.

Protocols and results - *(i) Methodological developments*. The amount of FC estimation errors in seed-based FC analyses was quantified through surrogate data analysis and two approaches for FC maps thresholding have been introduced in order to increase the reliability of single-subject FC analyses. Further, an automated denoising method (FMRIB's ICA-based X-noisefier - FIX), developed in collaboration with the FMRIB Centre (University of Oxford, UK), allowed to further improve the FC estimation as, through the cleaning of the raw single-subject data, it can be applied to any FC analysis. The cleaning procedure with FIX consists of the following major operations: single-subject spatial independent component analysis (ICA), component-wise feature extraction, classifier training, components classification, and removal of the artefactual

components from the data. FIX achieved over 95% classification (signal vs noise) accuracy for the training sub-sets built by hand-labelling the single-subject independent components in three different datasets. The procedure for artefact removal was then optimized, testing the efficacy of several cleaning options on different acquisition sequences (standard EPI and multi-band slice accelerated EPI) at two group ICA model orders (low- and high-dimensional ICA) by means of timeseries (timeseries amplitude and spectra), network matrices, and spatial maps analyses. FIX is now publicly available (www.fmrib.ox.ac.uk/fslwiki/fsl/FIX) and, partly due to the study performed in this thesis, FIX is now in use as part of the default analysis pipeline in the Human Connectome Project (HCP, http://www.humanconnectomeproject.org/); already over 200 subjects' worth of hour-long datasets having been released to date and FIX-cleaned data is the recommended version of the RS-fMRI data that is publicly available.
(ii) Applications. The impact of different data-driven cleaning approaches for RS-fMRI data was evaluated on a population of aged healthy controls and patients with mild to moderate AD. Among the tested approaches, the cleaning procedure with FIX showed to be the most effective in correctly detecting the typical FC alteration of the DMN in AD patients. Finally, we obtained promising results for a better localisation and quantification of FC alterations in AD on two RSNs of interest (DMN and sensory-motor network),

through the combination of an effective cleaning procedure and high-dimensional ICA decomposition, supported by a component classification based on low-dimensional ICA. The results of the spatial and temporal RSNs analyses showed that the study of the temporal information and the more detailed parcellation of the RSNs of interest were able to reveal FC changes in AD that were not detectable with the more common approach of low-dimensional spatial map analyses.

Discussion and conclusion - The present work has demonstrated and validated both the optimization of known protocols and also novel approaches in basically two directions: 1) an effective cleaning of RS-fMRI data for reliable FC analyses; 2) a more detailed parcellation of the brain and the analysis of the temporal information with timeseries and network analyses.
With a preliminary study, the amount of FC estimation errors in one of the most common FC analysis techniques (seed-based FC) was quantified and a thresholding method was proposed for a reliable single-subject FC analysis. Through the development of FMRIB's ICA-based X-noisefier (FIX), we then demonstrated that, by combining an accurate ICA component classifier with an effective approach for noise removal, we were able to remove artefacts directly from the raw data, automatically, and that we were not removing significant amounts of non-artefactual signal. Moreover, with multiband accelerated

sequences and effective cleaning, we were able to perform higher dimensionality decompositions and more detailed RSN analyses than with a standard EPI acquisition. The proposed denoising approach was also demonstrated to be particularly beneficial in clinical applications, as it allowed to correctly detect FC alterations in mild to moderate AD patients. Finally, we showed that high-dimensional ICA, supported by a component classification based on low-dimensional ICA, could be successfully applied in clinical studies (e.g. in AD) to gain additional knowledge regarding brain FC changes in diseased populations. A detailed parcellation of the brain and the analysis of the temporal information (e.g. amplitude and networks analyses) could give further and earlier insight into the detection of functional connectivity alterations in pathological conditions and their monitoring at different stages. The promising results obtained in describing the functional disconnections due to this neurodegenerative disease support further efforts in this investigation direction, towards the definition of reliable non-invasive biomarkers for AD.

MINING MEDICAL DATA TO DEVELOP CLINICAL DECISION MAKING TOOLS IN HEMODIALYSIS: PREDICTION OF CARDIOVASCULAR EVENTS

Jasmine Ion Titapiccolo - Supervisor: **Prof. Maria Gabriella Signorini**

During the last few decades the habit of recording electronic medical data has greatly spread, thus machine learning is likely to play an increasingly larger role in clinical settings. The possibility to storage huge quantity of medical data and so the growth of medical databases is really crucial since it allows to search for interesting information hidden in the data. Machine learning techniques can be very useful to search for patterns and relationships between medical variables and patients patho-physiological states, thus computer scientists are increasingly applying these techniques to clinical data. Prediction of specific events is one of the main goals of machine learning: the application of machine learning techniques in preventive medicine can lead up to the identification of some factors that can be predictive of risky situations. Chronic hemodialysis (HD) patients experience a very high mortality, which is about 20% per year, and chronic renal failure has recently been defined as a "vasculopathic state" since cardiovascular deaths among dialysis patients are approximately 30 times higher than in the general population. The understanding of factors involved in cardiovascular events insurgence among these patients is currently a clinical important

target of nephrology care. Some attempts have been recently made to predict outcomes in dialysis patients but the involved phenomena are very complex: an accurate prediction of patient course is still very challenging. Purpose of this PhD thesis is to predict hemodialysis course in terms of cardiovascular events using a real dataset extracted from EuCliD system, collecting dialysis treatment and patients data routinely collected in the clinical practice. Information about more than 4500 patients treated for 18 months, three time per week by HD was inspected. Incident HD patients, i.e. patients in their first 18 months of HD treatment, were examined for the high prevalence of cardiovascular diseases during the dialysis starting period. Not overlapping six months in length temporal windows were identified and predictive models of cardiovascular events insurgence in the next temporal window were built. The implemented predictive models where built on variables extracted from the current six months temporal window: thus the insurgence of cardiovascular events in months7-12 was predicted using variables computed on months 1-6 (TW1/TW2 model) and cardiovascular events insurgence in months 13-18 was predicted using variables computed on

months 7-12 (TW2/TW3 model). A dataset composed by 39 physiological and treatment variables was computed and used in the analyses. Preprocessing techniques included standardization of variables, handling missing data through mean values imputation and class balancing through minor class oversampling with replacement.
Chosen machine learning techniques belong to both classification and clustering based methods. In the classification methods Lasso logistic regression, random forest and support vector machines can be found. On the other hand, regarding the clustering based approach, supervised self organizing maps (SOM) were chosen. Each model was deeply investigated to better understand the physiological mechanisms going on in a sudden deterioration of hemodialysis patient's cardiovascular system. Logistic regression coefficient were computed to better understand the influence of variables values on cardiovascular events insurgence. Known the complexity of the underlying physiological phenomena and the presence of strong non-linear relationships among the involved variables and cardiovascular outcome, the random forest method was

chosen. Random forests are able to identify and exploit in the prediction the non-linear patterns hidden in the data, thus this method was selected and compared to logistic regression in terms of predictive performance. The best predictive performance was indeed obtained through random forests which showed an AUC of the ROC curve equal to 73% and sensitivity higher than 70% in both the temporal windows, proving that they are able to exploit non-linear patterns retrieved in the feature space. The investigation of the obtained random forest models through the analysis of out of bag (OOB) variable importance values allowed a better understanding of specific patho-physiological conditions placing patients to a higher cardiovascular risk. Linear kernel SVM was implemented too: a similar performance to logistic regression was obtained. Based on a clustering approach supervised SOM were chosen mainly because their visualization is able to present both similarity between positive and negative correlated variables as well as non-linear relationships hidden in the data. Nevertheless SOM model showed a predictive performance similar to logistic regression, thus lower than random forest performance. The dissertation also deals with feature selection since two wrapper strategies were embedded in the built models to identify subsets of features with the best prediction effectiveness. In particular backward wrapper strategies based on random forest variable importance ranking of variables and on minimum redundancy maximum
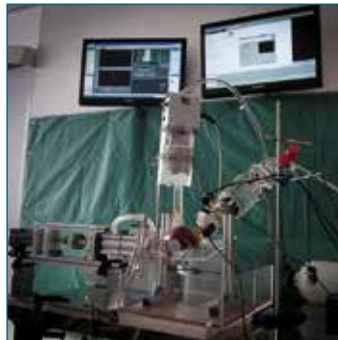
relevance (mRMR) ranking of variables were implemented. Predictive capability of random forest models was increased through the variable importance based wrapper and effective subsets of 6 variables (mean albumin percentage content, percentage loss of body weight in months 1-6, total protein content, mean C-reactive protein content, mean creatinine content and age of patients) in TW1/TW2 model and 4 variables (mean albumin percentage content, total protein content, mean C-reactive protein content, and mean value of diastolic pressure measured after HD administration) in TW2/TW3 model were identified. In this way more interpretable models having an optimized predictive performance were obtained: AUC of the ROC curve resulted to be equal to 77.1±2.9% and 74.8±3.4% respectively. On the other hand predictive capability of SOM models was increased through mRMR wrapper strategy. Subsets composed by 17 and 24 variables were obtained in TW1/TW2 and TW2/TW3 models respectively. Predictive models built on the selected subsets of features showed a better performance than the model built on the entire set of variables giving AUC of the ROC curve equal to 67.2% (standard error: 4.1%) and 64.4% (standard error: 4.0%) respectively. SOM models were chosen and broadly investigated because they give the opportunity to further and efficiently investigate the relationships between the variables involved in the prediction. Moreover using the supervised approach it is possible to identify on the final map the

region of neurons voting for patients having cardiovascular events in the next temporal window. In this way particular feature patterns of patients affected by cardiovascular disease in the next future could be easily identified.
Getting insights in the implemented models and through the analysis of the identified nested subsets of features it was possible to notice that the presence of an inflammation status, malnutrition or a not proper ultra-filtration of the patient through dialysis treatment are significant predictors of cardiovascular events insurgence in incident HD patients. These factors highlight an increased risk of cardiovascular system disruption: personalized therapy strategies can be devised to lower the cardiovascular risk in incident HD patients.

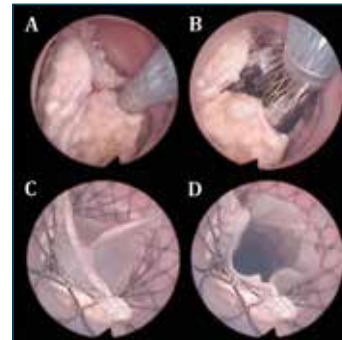# MULTIFUNCTIONAL PASSIVE-HEART PLATFORMS FOR IN VITRO HEMODYNAMIC STUDIES

Alberto Maria Leopaldi - Supervisor: **Prof. Alberto Redaelli, Prof. Gianfranco B. Fiore**

In recent years, the need for realistic *in vitro* models of the cardiovascular system has become more stringent due to the substantial changes of the clinical approaches to cardiovascular pathologies towards reparative, minimally-invasive and transcatheter techniques. For most of such applications, the interaction between implanted device or repaired structure and *in vivo* environment is not strictly limited to the hemodynamics, but involves anatomical and functional aspects that are crucial for the outcome of the procedure. Recently, researchers addressed this issue developing mock loops in which entire hearts could be housed. Most of such designs tried to maintain cardiac contractility *ex vivo* through myocardium perfusion, and were capable of reproducing the physiological ventricle pressure-volume relationship. Nonetheless, the complexity and costs of the related experimental protocols represented serious drawbacks. Alternatively, Richards et al. suggested to use the heart as a passive structure, dynamically pressurized by an external pulsatile pumping system, thus significantly reducing both complexity and cost. The aim of this study was to develop novel multifunctional *in vitro* passive-heart platforms that can represent effective


1. picture of the first passive-heart platform that was developed.

2. representative endoscopic images obtained during a TAVI procedure performed on the passive-heart platform with the use of a pathological model.

tools for research, device testing and training purposes and reduce the need for *ex vivo* and animal models. The first system (Figure 1) was inspired by the work of Richards et al., as its functioning principle consisted in the cyclic internal pressurization of the left ventricle by means of a piston pump connected to the heart apex. The design of our mock loop was aided by an *ad-hoc* defined lumped parameter model, that was used as a predictive tool and led to the achievement of hemodynamic conditions that closely mimicked the physiological ones. The system also showed excellent imaging capabilities, and good valve function. As a main drawback, the actuation methodology caused a paradoxical motion

of the ventricular walls during the cardiac cycle, which, however, didn't impair the mitral valve competence, and an altered fluid dynamic field inside the left ventricle. The passive-heart platform concept was shown to be perfectly suitable for performing realistic *in vitro* studies of TAVI applications (Figure 2). Our aim was to simulate *in vitro* the typical scenario in which TAVI procedures are performed *in vivo*, so to provide physicians with a multi-functional tool that could be used for both training and research purposes. To achieve this goal, the passive-heart system was optimized with improved design solutions, that led to the development of a simple, reliable and cost-effective system capable of closely
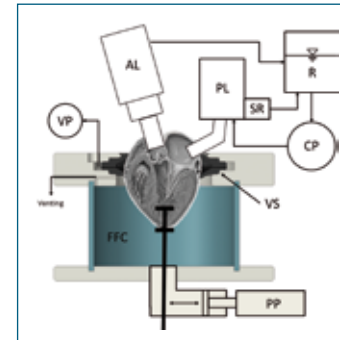
3. schematic of the second passive-heart platform, i.e. the one in which the ventricles are externally activated.

simulating the hemodynamic and anatomo-morphological *in vivo* environment. The system was successfully used to perform TAVI under fluoroscopic guidance and simultaneous intracardiac visualization in a catheterization lab. An *in vitro* model of aortic stenosis was also developed, assessed, and used in the simulated TAVI procedures to better mimic the pathological scenario. We then tackled the development of another passive-heart mock loop, that adopted a complementary working principle as compared to the first passive-heart setup in order to better simulate the physiological behaviour of the ventricles avoiding oddities in the ventricle wall motion. Indeed, this platform mimicked the pulsatile pumping function

of the left heart through the cyclic external pressurization of the ventricular walls. The system (Figure 3) was capable of reproducing physiologic hemodynamic conditions, and allowed for endoscopic imaging of the cardiac structures. Anyhow, differently from the former passive-heart approach, this actuation methodology induced mitral valve prolapse at high stroke volumes, due to the absence of papillary muscle contraction. The system was also used to perform a pilot study, in which the acute post-operative scenario after the implantation of a cf-LVAD was simulated, and the AV function for different levels of support was analysed. Our results were in line with clinical observations and previous studies, and the acquisition of high-speed video recordings of the aortic valve allowed deeper insights into the kinematic and morphological alterations that cf-LVAD may induce on the AV function. Finally, we described the first steps that have been made towards the development of a 4-chamber closed-loop mock loop for entire hearts, being either passive hearts actuated by model-controlled pumps, or isolated beating hearts. An existing hybrid 4-chamber system, developed at the TU/e, was redesigned to substitute the set of hydraulic components

modelling the heart function with the real heart structure that will be used instead. Thus, an anatomical study was conducted to develop an interface allowing the connection of the main heart vessels to the mock circulatory loop preserving their physiologic configuration. The mock loop was then redesigned accordingly, also considering the requirements related to the performance of *ex vivo* experiments. In conclusion, this thesis elucidated the role played by passive-heart mock circulatory loops in the broad panorama of the experimental platforms for cardiovascular research. Given the results of our research, we believe that in the near future passive-heart platforms may become the choice of election among the *in vitro* platforms for many applications, potentially reducing the need for *ex vivo* and animal models. Furthermore, they might represent an interesting option for physicians training and protocol optimization, owing to the tremendous awareness that the operator experiences when simulating surgical/interventional manoeuvres with these laboratory apparatuses.

METHODS FOR THE ANALYSIS AND INTERPRETATION OF THE CYCLIC ALTERNATING PATTERN OF SLEEP

Sara Mariani - Supervisor: **Prof. Anna M. Bianchi, Prof. Mario G. Terzano**

The study of the Cyclic Alternating Pattern (CAP) is an approach of relatively recent introduction integrating the traditional analysis of the sleep architecture (*macrostructure*), that classifies night recordings into REM and NREM N1, N2 and N3 stages, with a more detailed description of the dynamic mechanisms at the basis of sleep (*microstructure*). CAP is a periodic activity visible on NREM sleep Electroencephalogram (EEG), composed of a higher activation phase, called phase A, and a phase in which only the background is visible, called phase B. Both A and B phases have a duration between 2 and 60 seconds. The spectral content of phases A allows their classification into three subtypes: A1, dominated by high-voltage delta waves (0.5-4 Hz); A2, when rapid activities occur for 20-50% of the total activation time; c) A3, characterized by rapid activities, especially beta (15-30 Hz), that occupy more than 50% of the total phase A duration.
Sleep microstructure can be accompanied by Heart Rate Variability (HRV) modifications, and arousal instability during CAP is associated with a concomitant activation of the autonomic parameters, i.e. cardiorespiratory rate and muscle tone during phase A and with their attenuation during

phase B.
Despite being a physiological component of the sleep structure, CAP is also a reactive phenomenon, and increments of CAP rate, obtained as the ratio between NREM CAP sleep and total NREM sleep, occur in situations of sleep disruption, such as insomnia, depression, sleep apnea syndrome, periodic limb movements, epilepsy. In the light of this, CAP contains information that is relevant in clinics for evaluating the quality of sleep and helping the study of sleep-related disorders.
At the present time, however, CAP analysis is restricted to a limited number of sleep laboratories, due on one hand to a certain criticism towards this phenomenon, considered very descriptive and dependent on the scorer, and on the other hand, to the practical difficulty in performing its scoring, based on the visual recognition of each phase A on the EEG of whole night sleep recordings, requiring specific skills and knowledge and representing a very time-consuming activity.
This dissertation aims to address these issues by providing a set of instruments to allow a quantitative and objective characterization of CAP, making its study more approachable in everyday clinics. It focuses on giving a mathematical description of the features that

characterize phases A of CAP, quantifying the underlying physio-pathological phenomena. At the same time, it has as its goal the implementation of an automatic method to detect activations that may constitute phases A, with the aim not only to accelerate and optimize the physician's time, but also to provide a more precise and objective detection based on EEG parameters.
The first study presented in this thesis focuses on the identification of quantitative distinctive EEG features characterizing the A phases of CAP. Nine descriptors are computed: six band descriptors (low delta, high delta, theta, alpha, sigma and beta), the Hjorth activity in the low delta and high delta bands and the differential variance of the EEG signal. The information content of each descriptor in recognizing the A phases is evaluated through the computation of ROC curves. The results show that it is possible to attribute a significant quantitative value to the information content of the descriptors, giving a mathematical confirmation to the features of CAP, generally described qualitatively.
These parameters are then employed to train a *machine learning* tool for the automatic scoring of CAP phases A. The annotations provided by an

expert clinician are used as gold standard and four alternative mathematical classifiers were implemented: 1) discriminant function, 2) Support Vector Machines (SVMs), 3) Adaptive Boosting (AdaBoost), and 4) supervised Artificial Neural Networks (ANNs). The results of the classification show average accuracies equal to 84.9% and 81.9% for the linear discriminant and the SVMs respectively, 79.4% for AdaBoost and 81.5% for the ANNs.
The remaining weaknesses of the method, i.e. the presence of some false positive detections, and the difficulty in determining the correct duration of the recognized phases A, are then addressed by three alternative approaches. The first focuses on re-computing the descriptors on windows of variable length selected on the EEG by means of a segmentation technique based on the Spectral Error Measure. The new descriptors show a higher information content with respect to those computed on windows of fixed length, and are used to train a linear discriminant for phase A classification. The final accuracy is equal to 86.09%, on average. In addition, it is shown how a completely automatic CAP detector, independent from any human assistance, can be obtained by including in the system an automatic NREM sleep isolation, with good reliability (accuracy=91.18, Cohen's Kappa=0.9).
Another approach examines the possibility of increasing the specificity of the method by introducing a second EEG lead and combining the resulting classification vectors obtained

employing the two sets of descriptors extracted from the two leads, and used to train SVMs for A phase detection, by means of simple logical principles. The results show an average accuracy of 83.84% and an average Cohen's Kappa of 0.50.
The last method focuses on the correct determination of the duration of the recognized phases A. It shows that starting from a well-defined point inside a phase A it is possible to find the exact borders of the phase by means of a statistical comparison of the variance of the EEG on partially overlapped windows. The results show that A1 and A2 phase borders can be detected with good accuracy, while further effort must be put in refining the identification of A3-subtype phase borders.
It must be highlighted that, although these three approaches are conducted separately in order to evaluate singularly which measures could be effective in increasing CAP detection accuracy, the techniques presented in each of them can naturally be combined to obtain a reliable and efficient classifier.
The characterization of CAP is completed with its study in relationship to the Autonomous Nervous System, which highlights linear correlations between the A1 index and variance-quantifying or spectral HRV parameters, and nonlinear correlations between CAP time and A1, A2 and A3 indexes and linear time-domain and spectral HRV parameters in healthy subjects, allowing a quantitative confirmation of the global vagal-promoting role of phases A1 while at the same time showing

an existing although less marked sympathetic-promoting role of phases A3 in absence of pathology. Also, the presence of a relationship between fractal scaling in cardiac activity and the presence of CAP is suggested. The second important result of the same study is the possibility of employing global CAP and HRV parameters, either singularly or combined, for a distinction among classes of patients affected by different sleep-related disorders.
This dissertation ends with the presentation of the CAP Sleep Database for PhysioNet, containing all the recordings employed in the featured studies, annotated for the macro- and microstructure. Sharing these data will allow other research groups to contribute to the final goal of developing an automatic classifier, triggering a virtuous circle for CAP studies, both from a clinical and a bioengineering point of view. A further key strength of the data being publicly available is the possibility of reproducing all the above mentioned studies and any future studies employing the same dataset, crediting them with a more robust scientific credibility.
It can be concluded that the work presented in this thesis may constitute a valid platform for the development of a complete, automatic CAP classifier, addressing the problem of lengthy visual scoring and inter-scorer variability. At the same time, it gives objective credibility to this phenomenon suggesting tools for its physiological interpretation and application in clinical practice.

¹⁹F MRI AND MOLECULAR IMAGING: OPTIMIZATION AND APPLICATION IN MULTIMODAL IN-VIVO BRAIN CELL-TRACKING

Alfonso Mastropietro - Supervisor: **Baselli Giuseppe**

Scientific Background

Today's diagnostic imaging techniques are found on betraying alterations on anatomy and morphology at microscopic level, sometimes overlapping functional information, in order to characterize a disease. Molecular imaging (MI) takes a central place in the functional and metabolic imaging paradigm and allows early detection of changes related to a pathology on a molecular level, using disease-specific probes. In the last few years, ¹⁹F MRI has gathered growing importance in *in vivo* bio-molecular and cell-tracking studies, thanks to desirable properties of ¹⁹F nucleus similar to the ¹H. For these reasons, ¹⁹F MRI can produce an image quality similar to ¹H MRI and a potentially high contrast to noise ratio, if a fluorinated compound can be used as contrast agent. On the contrary, the low sensitivity of MRI in general, the potential toxicity and the insolubility in water could generate some issues in the preparation and in the imaging of these molecular probes. A large number of studies has demonstrated that ¹⁹F MRI sensitivity is limited to mM concentration, so that MR is less sensitive than nuclear medicine techniques for in vivo applications. For this reason increasing sensitivity is the main goal for the development of ¹⁹F MRI.

Rationale and Aim of the thesis

The main aim of this work was to develop and apply simple numerical methods to optimize MR sequences in order to improve sensitivity for ¹⁹F MRI studies in high field scanners (7T, 11.7T) on phantoms containing fluorinated compounds in different biological environments. Furthermore, an *in vivo* application of ¹⁹F MRI combined with BLI is presented in order to assess the fate of implanted human Neuronal Stem Cells (hNSCs).

Materials and Methods

Numerical simulations were developed based on the Bloch equations in order to estimate the best parameters settings for improving SNR in ¹⁹F MRI. Images were acquired on high field MRI preclinical scanners (7T or 11.7T). Different RF double tunable (¹H/¹⁹F) coils were used in this study (linear volume RF coil and surface coils). Different fluorinated compounds were used in different biological environments (hexafluorophospate KPF_6, Triflurorotoluene TFT, Fuorodeoxyglucose FDG, Cell Sense 1000). Relaxometry studies were carried out in order to estimate the actual relaxation times of ¹⁹F compounds.

SNR from experimental MR images was compared to the simulated one for different pulse sequences (RARE, FLASH, unbalanced SSFP). In order to perform a multimodal imaging ¹⁹F MRI/BLI study, transgenic human Neuronal Stem Cells (hNSCs) expressing Luc2 were labeled with ¹⁹F perfluorinated compound (CellSense-1000, USA) and injected in brains (striatum) of nude mice. Animals were followed for 1 week then sacrificed for immunohistochemistry (IHC). BL images were analyzed using a novel framework developed to perform an automatic segmentation of images in order to distinguish the noise, the background and the signal. Signal to Background Ratio was proposed as figure of merit to evaluate cellular viability.

Results

Numerical simulations were proven to be a useful tool to optimize image sequence parameters for increasing SNR in RARE sequences. In figure 1 the optimal parameters (echo train length ETL, and repetition time TR) are displayed with and without driven equilibrium (alias flip back, FB) pulse (FBON and FBOFF, respectively). As clearly shown in figure 1a and 1c, ETL increases according to the increase of T_2 for both sequences. Considering FBOFF,
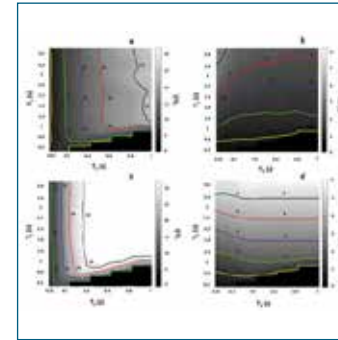

1. Optimal parameters maps (ETL and TR) are shown in parametric space for different T_1 and T_2. RARE sequence optimization with driven equilibrium pulse (a,b) and without (c,d) is displayed. A wide range of relaxation times T_1 (0.2-4 s) and T_2 (0.05-1 s) was considered. In this simulation a TE of 11.6 ms was chosen.

the optimal TR is almost proportional to T_1 (figure 1d). Conversely, with FBON the dependence of the optimal TR vs. T_1 is not trivial (figure 1b). Experimental validation of the optimization protocol has shown a mean error between simulated and experimental SNR less than 10% and a maximum error of about 20%. A comparison between GRE sequences was also performed. In general FLASH sequence has the worst performance compared to RARE and unbalanced SSFP. Non-spoiled GRE highlighted an increase of SNR up to 65% compared to RARE sequence and up to 517% compared to spoiled GRE. RARE sequence has an increase of SNR up to 73% compared to spoiled GRE. Hence, optimized RARE was used for *in-vivo* experiments. *In vivo* application of ¹⁹F MRI was performed in order to evaluate the fate of hNSCs implanted in the mouse brain. ¹⁹F MRI allows to non-invasively localize and quantify cells in the tissue

volume, as shown in figure 2. A progressive reduction of the number of cells was highlighted for both cell lines in a longitudinal study. This result was in a good agreement with the decrease of signal in BLI. The presence of a massive infiltration of macrophages can explain the reduction of cell viability after transplantation as highlighted by immunohistochemical analysis.

Discussion

In this work, the optimization of fast sequences such as RARE and GRE was addressed, in order to increase this sensitivity and SNR, both through numerical simulations and experimental validation. The substantial agreement between simulations and experimental results supports the correctness of the approach. We proposed a useful method to optimize RARE sequence considering the actual relaxation times of the fluorinated compounds in different environments. Even if this approach is based on *in vitro* or *ex vivo* studies, it can be generalized also for *in vivo* studies; however, an optimization of relaxometry procedure is needed to limit time to *in-vivo* constrains.. The optimization process can improve SNR up to 50% by tuning both TR and ETL. As to detection threshold, by an optimized RARE sequence on a 7T MRI scanner, using a linear volume coil, with an acquisition time of two hours, a threshold of 6.22 10^{16} fluorine atoms per voxel was shown. On a 11.7 T MRI scanner and with a surface RF coil the sensitivity threshold was reduced to about 10^{15} fluorine atoms per voxel. In this case a low number of cells can
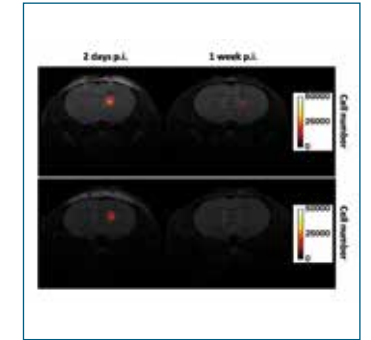
2. Quantification of 19F labeled cells obtained from *in vivo* studies. Two different animals are displayed. Injected hNSCs were clearly detected by 19F MRI and superimposed on anatomical high resolution 1H MR images. Images were acquired 2 days and 1 week post injection

be detected in *in vivo* studies (about 10^4 cells per voxel). Thus, the optimization protocol proposed and validated for Cell Sense labeled cells can be used to improve sensitivity for ¹⁹F MRI cell tracking studies. ¹⁹F MRI was used to localize transgenic hNSCs *in vivo* after implantation in the mouse brain. The use of ¹⁹F MRI allows to evaluate the efficacy of the implantation with a noninvasive and quantitative approach. The combination of ¹⁹F MRI and BLI is an interesting approach in order to obtain functional and morphological information.

IMAGE-BASED ANALYSIS OF REGIONAL LUNG FUNCTION

Francesca Pennati - Supervisor: **Andrea Aliverti**

The recognition that lung disease is non-homogeneous and that local parenchymal alterations can exist before global measurements of lung function begin to deteriorate have lead to the development of multiple imaging techniques for the quantification of regional changes in lung structure and function. Clinical applications of the regional assessment of lung function include detection of early signs of disease, further insight into the progression of disease, planning pulmonary interventions and evaluating parenchymal alterations induced by therapy. A number of imaging techniques have been proposed to regionally quantify lung function and identify local ventilation defects in humans, as nuclear medicine, hyperpolarized gas magnetic resonance and computed tomography using xenon gas. Nevertheless, several issues have prevented their routinely clinical use, including long scan time, low resolution, high costs, and/or low accessibility. In the last decade, new methods based on standard computed tomography (CT) scans acquired at different lung volumes have been proposed as surrogates for regional ventilation in health and disease.

The aim of the thesis was to investigate the use of standard computed tomography (CT) and magnetic resonance imaging (MRI) acquired at multiple lung volumes in combination with deformable image registration to identify any local heterogeneity related to physio-pathology.

To this aim we introduced and evaluated a new straightforward method for registering pulmonary CT images acquired at different inflation volumes. Pulmonary image registration is challenging because of the unique structure of the lung, its high deformability and non-uniform intensity changes during breathing. We proposed a new approach to pulmonary image registration, based on the reconstruction and the combination of the main pulmonary structures, i.e. vessels, fissures and external lung surface to modify parenchymal intensity prior to the application of the registration algorithm. The algorithms, applied to both four dimensional CT and high resolution CT acquired during tidal breathing and in breath-hold at total lung capacity and residual volume, demonstrated an increased accuracy of the results with the application of the pulmonary structure enhancement.

The method was first investigated in a group of healthy volunteers acquired in breath-hold at residual volume and total lung capacity in order to compare three CT-based surrogates for regional ventilation, namely changes in density (ΔHU), specific volume (sVol) and specific gas volume (ΔSVg), in relation to the physiological determinants of the non-homogeneous distribution of ventilation, namely gravity dependence and position along apex-base direction. Results demonstrated that the three parameters behave differently only along the gravity direction with specific gas volume variations not influenced by gravity, demonstrating that the amount of gas relative to mass of tissue delivered to alveoli is quite constant along ventro-dorsal direction. The heterogeneity of specific gas volume is therefore the result of phenomena other than gravity, thus more reliable in discriminating pathological and healthy regions and is likely to decrease variation in longitudinal studies.

This heterogeneity was investigated in the diseased lung, in relation to tissue destruction and collateral ventilation in severe emphysema. Defined as a 'condition of the lung characterized by abnormal enlargement of the air spaces distal to terminal bronchiole, accompanied by destruction of alveolar walls' emphysema leads to small airways collapsing during forced exhalation, resulting in airflow limitation and gas trapping in the lungs. Recently, as alternative to invasive and expensive surgical treatments for the treatment of severe emphysema, different bronchoscopic techniques have been introduced such as airway bypass, endobronchial one-way exit valves, thermal vapour ablation, biological sealants and airway implants. Functional regional analysis of the lung is required as a tool for planning and guiding these treatments. To study regional lung function and characterize regional variations of density and specific gas volume (SVg) in disease, a group of healthy and severe emphysematous subjects were scanned via HRCT at residual volume and total lung capacity and any heterogeneity of lung function was explored in relation to gravity and collateral ventilation. Results demonstrated that both Hounsfield Units and specific gas volume variations were able to discriminate healthy from severe emphysematous lung and to qualitatively and quantitatively evaluate lung function. These results have important clinical implications in the assessment of different stages of disease, and in the evaluation of pharmacological or surgical treatments, such as minimally invasive interventions. Once translated into clinical practice may be helpful to identify regions (lobes and/or segments) where gas trapping is more pronounced and to distinguish those patients with and without collateral ventilation and therefore who are more or less likely to benefit from lung volume reduction by minimally invasive interventions.

In a further study lung heterogeneity was investigated in relation to within-breath re-distribution of ventilation in tumor lung. Recent advances in pulmonary imaging suggest that incorporating a local description of lung function into lung cancer treatment planning would reduce radiation-induced damage of normal lung tissue. Thus, we regionally investigated the distribution of pulmonary function during free breathing in patients with lung cancer on the basis of specific gas volume changes. The study demonstrated that the tumor introduces a change in the distribution of ventilation in the surrounding region, but does not alter the relative contribution of the other regions, suggesting that areas of lung parenchyma with normal function could be identified as organs at risk during radiotherapy treatment planning, thus reducing complications of normal pulmonary tissue.

In the end we investigated the feasibility of using multi-volume proton-MRI for a non-contrast assessment of regional lung function, by straightforward pulse sequences and hardware and without ionizing radiation. The study demonstrated that proton-signal changes between different lung volumes are in good agreement with 3He-ventilation imaging and can be successfully applied in both health and obstructive lung disease, being quite sensitive to ventilation non-homogeneities due to gravitational dependence and regional abnormalities resulting from obstructive lung disease. We think proton-MRI is likely to emerge as a new clinical and research tool to identify regional structure-function relationships with no need for special equipment and with no ionizing radiation.

At the conclusion of the work, we foresee the application of the present methods to the everyday clinical practice for the regional investigation of the lung in relation to pathology and response to treatment.

THE STUDY OF WHITE MATTER MICROSCOPIC DAMAGE IN NEURODEGENERATIVE PATHOLOGIES: ATLAS-BASED AND FMRI-GUIDED TRACTOGRAPHY

Marco Piola - Supervisors: **Prof. Monica Soncini, Prof. Gianfranco B. Fiore**

Rationale of the study

Saphenous vein (SV) graft disease represents an unresolved problem in coronary artery bypass grafting (CABG). After CABG, a progressive remodeling of the SV wall occurs, possibly leading to the lumen occlusion. This process is termed intima hyperplasia (IH). The investigation of cellular and molecular aspects of IH progression is a primary endpoint toward the generation of occlusion-free vessels that may be used as 'life-long' grafts.

Aim of the study

The aim of the present doctoral project was to explore new tools and procedures to investigate *ex vivo* the effects of altered mechanical load experienced by the human SV after CABG surgery. Advanced bioengineering/biotechnology modeling and prototyping tools, complying with biological methods and tissue engineering/ regenerative medicine requirements, were applied. Furthermore, the application of principles and methods of lifescience engineering were used for providing a reliable model system, facilitating the understanding of pathogenesis of vein graft IH. The integration of these methodologies led to devising a novel laboratory-oriented culture platform, that was used for conducting

extensive arterialization conditioning campaigns with human SVs, under strictly controlled hemodynamic conditions. In our view, this is crucial to obtain a global comprehension of disease progression, and in perspective to perform comparative studies of drug administration or gene expression modulation, to devise preconditioning protocols and/or regenerative medicine strategies that reduce the clinical impact of vein graft pathology.

Design of a novel *ex vivo* vessel culture system (EVCS)

The EVCS is designed to apply a CABG-like pressure stimulation (CABG-PS, pulsed pressure 80-120 mmHg), or a steady flow perfusion, (VP, 5 mmHg*)* within a controlled and strictly reproducible mechanical environment. During culture, SV grafts are hosted in a culture chamber accommodated inside an incubator. The chamber is connected to a hydraulic circuit and actuators to apply pressure stimulation to the human vessels. The hydraulic actuators are managed by a programmable monitoring and control system, which operates via a pressure-based feedback loop. Functional tests were performed using SV samples, in order to validate the robustness and the reliability over time of the control system, and to

verify the sterility maintenance. The outcomes of these tests indicated a good reliability of the control system, and the maintenance of a sterile environment provided by the EVCS, suitable for stimulation experiments and ensuring the SVs survival.

Current upgrading of the EVCS for a better biomimicking

Novel biomimetic features were introduced into the existing devices. The first upgrade version included a separated environmental control for the intra-luminal and extra-luminal culture media, giving the possibility to expose the intimal and the adventitial layers to distinct conditions (e.g. *hypoxia* of the adventitial layer, while blood-like oxygen condition of the lumen), thus mimicking the real status of CABG vessels *in vivo*. The regulation of the oxygen tension was attained by means of a purpose-developed de-oxygenation module, whose dimensioning was carried out by combining mathematical modeling and experimental design using dissolved oxygen probing within the conditioned culture medium. Functional tests were performed for characterizing the de-oxygenator loop. The obtained results demonstrated that the de-oxygenator module is a usable

alternative to unwieldy and expensive O2-controlled cell culture incubator. The bioreactor was finally upgraded to better replicate the full biomechanical stimuli involved in CABG arterialization (pulsatile wall stretch and wall shear stresses applied synchronously and with the correct phasing). Integrated with environmental control, this feature makes the device capable of applying complete CABG-like pressure/flow stimulation patterns to the hosted SV segments. Design efforts are dedicated to maintain strict engineering specifications concerning user friendliness, compactness, low-priming volume and low cost, while including the novel hydrodynamic features into the EVCS. Preliminary experiments were performed in order to analyze the flow and pressure traces. The results demonstrated the capability of the device of reproducing a fully biomimetic hydrodynamic mechanical stimuli involved in CABG arterialization.

An arterialization study of HSVs in the EVCS

An extensive arterialization campaign was finally conducted using the simplified version of the EVCS. Human surplus SV segments were subjected to VP or CABG-like pressure conditioning for a period of 7 days, and native SV segments served as control. After 7-days CABG-like pressure stimulation, the main findings were: i) distension and reorganization of the vessel wall components; ii) partial endothelial denudation, iii) smooth muscle cells rearrangement; iv) disarrangement of the vasa vasorum; v) decrease of SVs

wall thickness; vi) enlargement of the SVs luminal perimeter; vii) increased proliferation rate; vii) increased up-regulation of MMP-2 and basal level of TIMP-1 expression and ix) mechanoepigenetic mechanism involved in pro-pathologic commitment of SV-resident cells, particularly in cells located in the adventitia with SMCs progenitor characteristics. From a technical point of view, these results suggested that the EVCS is a suitable system for elucidating the mechanisms involved in the SV graft disease, within a controlled and strictly reproducible biomechanical environment. In fact, by providing a comprehensive level of monitoring and control over the biomechanical environmental, the *ex vivo* model provided the technological means to perform controlled arterialization studies aimed to understand which specific biological, chemical or physical parameters were involved in the SV remodeling. Concerning the mechano-biology, our findings demonstrated that the CABG-like pressure had an important role in the early events associated with the remodeling of the SV wall.

Conclusions and future directions

The use of a bioengineering approach to induce arterialization in cultured human SVs provided a valuable tools for studying the cellular and molecular pathways activated by exposure of the human SV to arterial-like conditions. The adopted strategy allowed to investigate *ex vivo* the effects of altered mechanical load experienced by the human

SV after CABG. The evolution of the *ex vivo* model of vein arterialization from a simple pressure-driven vessel straining system to a platform allowing vessel pharmacologic treatment under dynamic conditioning with/without application of arterial-like flow, will finally make possible to test targeting strategies against the selected effectors. The discovery of molecular targets (*miRNAs* and epigenetic traits) regulated by biomechanical/biochemical stimuli in the SV will produce an outstanding opportunity for the devise of novel translational protocols to reduce the consequences of IH. In this scenario, the customization of the conditioning platform to proceed with a tight control of arterialization process will be instrumental to achieve dynamic conditioning of the vein segments in the presence of drugs, which may interfere with the molecular progression of the pathology. In conclusion, the present project laid the basis for a potential translation from bench to bedside of graft preconditioning and pharmacologic treatments aimed at minimizing and reducing the clinical impact of vein graft disease in patients undergoing CABG. In this scenario, the developed *ex vivo* vessel culture system will be the advanced, safe, strictly controlled and reliable bioengineering tool that will permit autologous graft treatments preceding CABG surgery.

ENHANCING THE INFORMATION CONTENT OF BOLD FMRI DATA ANALYSIS THROUGH UNI- AND MULTI-MODAL APPROACHES

Roberta Sclocco - Supervisor: **Anna Maria Bianchi, Sergio Cerutti**

Although magnetic resonance imaging (MRI) was invented in the early 1970s, its use in cognitive neuroscience expanded greatly with the advent of blood oxygenation level dependent (BOLD) functional imaging, and by now functional MRI (fMRI) is a mainstay of neuroscience research. Standard analysis of fMRI data relies on a general linear model (GLM) approach to separate stimulus-induced signals from noise. In the GLM approach, the time course associated with each voxel is modeled as a weighted sum of one or more known predictor variables (e.g., the onset and offset of an experimental condition) plus an error term. The aim of the analysis is to estimate if, and to what extent, each predictor contributes to the variability observed in the voxel time course. This approach is nowadays the most popular among methodologists and neuroimagers, since it is: 1) conceptually simple; 2) an incredibly flexible tool; 3) implements the standard statistical testing framework; and, maybe the most important feature, 4) readily available in standard packages (SPM, FSL, Afni). Therefore, proposing approaches to unimodal and multimodal fMRI data analysis within the GLM framework appears to be particularly favorable, both for their

simplicity and their immediate applicability in the neuroimaging community.

The present dissertation is composed by three main sections. The first one is devoted to the integration between BOLD fMRI data and various biological signals. In fact, the recent technological advances allow now the acquisition of different measurements concurrently with fMRI, opening thus the way to multimodal integration analyses aimed at identifying the neural correlates of different physiological signals. In particular, the complementary features of electroencephalography (EEG) and BOLD-fMRI constituted the basis for recent developments in the integration of these neuroimaging modalities. One of the possible approaches combines EEG and fMRI measurements by using temporal- or frequency-specific information derived from EEG to obtain regressors of interest then used in the common GLM framework: this multimodal strategy is usually referred to as EEG-informed fMRI analysis and it differs from the classical fMRI analysis in its unique ability to selectively localize the fMRI correlated to specific neuronal events or rhythms. In particular, the issue of estimating the correct EEG-to-BOLD transfer function is addressed. In addition

to the investigation of the neural correlates of the EEG signal, one of the central outstanding questions, given the features of the BOLD signal, is whether and how autonomic nervous system (ANS) functions are related to changes in brain states as measured in the human brain. Several research lines have made important progress in showing that ANS functions such as cardiac pulsation, heart rate variability and skin conductance could be considered as a theoretically meaningful component of the signal that is useful for understanding brain function. This hypothesis holds particularly when studying cortical systems involved in regulation, monitoring and/or generation of ANS activity, such as those involved in decision making, conflict resolution and experience of emotions or aversive stimuli. Following this assumption, a growing number of studies have investigated the role of different cortical, subcortical and brainstem regions in autonomic control during a variety of different tasks and sensory stimuli, in order to identify the human central autonomic network (CAN). In the present work, sympathetic and parasympathetic correlates of nausea are explored (Figure 1). Finally, in addition to identifying the neural correlates of the autonomic response to a

specific stimulus, the ANS/fMRI approach can also be useful to explain unexpected responses to stimuli known to elicit a robust involvement of the autonomic system, as in the case of aversive stimuli like pain sensation. The ANS/fMRI analysis can offer in these situations a possible explanation for some observed "atypical" responses, allowing at the same time to link specific brain regions to the generation and the regulation of autonomic outflow to the examined stimulus.

In the second section, connectivity analysis of BOLD fMRI time series is addressed. The estimation of causal relationships within brain networks can be achieved by two main analysis approaches: model-based (e.g., structural equation modeling (SEM) and dynamical causal modeling (DCM)) and data-driven methods (e.g., Granger causality analysis or GCA based on vector autoregressive (VAR) modeling). Both approaches try to estimate directed causal influences between cerebral structures by extracting useful information from the temporal dynamics of signal that measure directly or indirectly neural activity from different regions/areas. The choice between GCA and DCM was recently widely debated in literature. On the one hand, GCA does not use any

biophysical model to account for the relationships between BOLD signal and neural activity; on the other hand, DCM needs strictly defined a priori hypothesis about connectivity structure. Even though these two different approaches are often described as opposites, it may be more constructive to think GCA and DCM as two complementary methods that try to explore connectivity, since they occupy different but equally important positions on a spectrum from "purely exploratory" to "purely confirmatory" methods. While DCM is fully implemented within one of the standard packages for fMRI analysis, GCA is usually carried out by means of ad hoc or already existing scripts adapted for the purpose. Therefore, an attempt was made to realize a specific toolbox for GCA on fMRI data, which was then applied to the study of epileptic seizure propagation. Finally, in the third section of the present work, a dynamical fMRI analysis is proposed for the study of the motor cortex hemodynamic modulation in an Unverricht-Lundborg (ULD) syndrome population. In ULD's disease, the most common progressive myoclonus epilepsy (PME) found in Europe, there is a common sensory cortex hyper-excitability, demonstrated by giant evoked potentials, accompanied
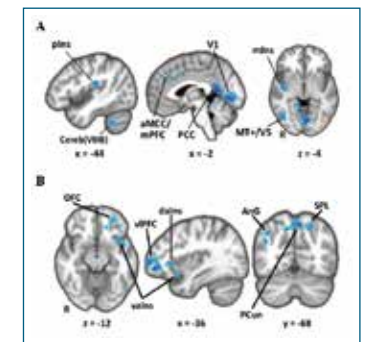
1. Parasympathetic (A) and sympathetic (B) correlates of nausea.

by defective sensorimotor integration. Despite the significant distortions in time and topographical distribution found in the alpha and beta bands synchronization/desynchronization (ERD/ERS) pattern, a recent fMRI study did not reveal any difference with respect to a control group with regards to activation intensity, latency or extent effects. Therefore, adopting a dynamical approach able to explore within-block modulation could allow to highlight differences otherwise invisible with a classical GLM analysis of a blocked design protocol.

TUMOR TRACKING IN PARTICLE THERAPY: DEVELOPMENT OF DEDICATED METHODS AND EXPERIMENTAL TESTING

Matteo Seregni - Supervisors: **Prof. Guido Baroni**

Introduction

Among the different radiation therapy techniques, particle therapy uses high energy ion beams to treat tumors with increased geometrical accuracy and enhanced biological effectiveness with respect to conventional photon radiation therapy. Considering thoracic and abdominal lesions, such advantages can be fully exploited only if inaccuracies caused by physiological organ motion (e.g. respiration) are compensated.

Tumor tracking is an effective motion compensation strategy currently applied in conventional radiotherapy: it consists in the real-time adaptation of the beam direction to follow the tumor motion along its trajectory. However, its application in particle therapy is challenging as the tumor motion interferes with the beam scanning path causing over- and under-dosages. In addition, radiological path length variations in the tissues traversed by the ions should also to be compensated by adjusting the beam energy.

In light of these premises, the aim of the proposed project is the development and the experimental testing of methods dedicated to motion modelling for tumor tracking in particle therapy.
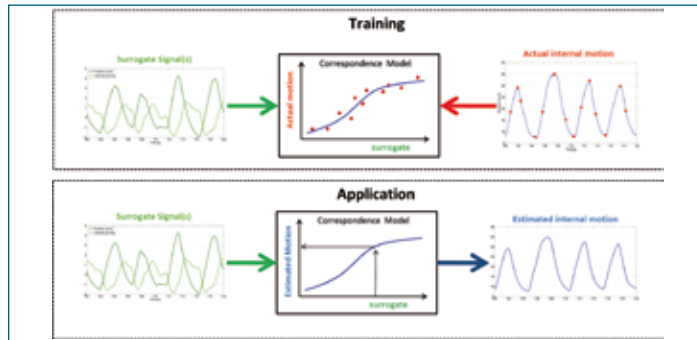
1. Surrogate driven tumor tracking general workflow.

Methods for tumor motion modelling

The proposed methods are in the framework of the external surrogate-driven tumor tracking (Figure 1). This approach involves the training of a patient-specific correspondence model describing the relationship between the internal tumor motion and the external surrogate signal, which can be acquired non-invasively and at high sample rate. The trained model is then used during irradiation to estimate the target position as a function of the surrogate.

The first tracking strategy that was investigated involved the application of machine learning methods for the fitting of the internal/external correlation function. State augmented polynomial models (SM) were considered as an extension of the current state of the art, represented by the CyberKnife® Synchrony RTS®. More complex models featuring specific generalization capabilities were also developed by means of Artificial Neural Networks (ANN) and Support Vector Regression (SVR). All the proposed models were tested individually as well as in a comparative study carried out on a Cine-MRI dataset, where the motion of multiple liver landmarks was recorded in five volunteers.

Average tracking errors were 1.34 mm, 1.43 mm and 1.32 mm for SM, ANN and SVR, respectively. No clinically relevant performance differences among the three models were observed. Such results were comparable with the spatial resolution of the cine-MRI data (1.29 mm).

A second tracking strategy was also tested: deformable image registration applied to time-resolved imaging (4D CT) was used to obtain a patient specific model able to estimate the entire CT volume corresponding to a given respiratory state. Such approach allowed to monitor the target position as well as the motion induced path length variation along the beam line. Retrospective studies on four lung cancer patients reported average geometrical tracking errors of 1.4 mm and water-equivalent path length differences between the estimated and the ground-truth volumes limited to 1.2 mm.

Experimental application of motion models in particle therapy

In the framework of the European Project ULICE (Union of Light Ion Centers in Europe), experimental activities were carried out at CNAO (Centro Nazionale di Adroterapia Oncologica, Pavia, Italy) and GSI (*Gesellschaft für Schwerionenforschung*, Darmstadt, Germany) to test the feasibility of correlation models-based tumor tracking in scanned particle therapy. The main objective was quantifying the accuracy of this motion mitigation strategy by means of dosimetric measurements in phantom studies.

The experiments relied on a robotic phantom to generate external (thorax) and internal (tumor) motion. A dedicated

2. Experimental set-up at GSI. The beam line (solid red line), the robotic phantom and the optical tracking system TVCs are highlighted.

optical tracking system used two IR TV-Cameras to monitor the thorax motion (Figure 2), providing the inputs for two correlation models (SM and ANN), which estimated the target position in real-time. According to these estimations, tracking correction vectors were computed and applied to the beam relying on steering magnets.

The dose absorbed by the target was measured using an array of ionization chambers during static (reference), compensated (tracking) and uncompensated irradiations. The dosimetric differences measured when tumor tracking was performed showed a statistically significant difference with respect to the measurements concerning the uncompensated irradiation, proving the potential effectiveness of tumor tracking

based optical motion monitoring and correlation models.

Conclusions

Different motion modelling methods were investigated. All the proposed strategies were proved to achieve tracking errors lower than 1.5 mm and therefore can be considered feasible methods for tumor tracking. Considering particle therapy, the dedicated experimental activities proved that respiratory motion can be effectively compensated by optically driven tumor tracking based on internal/external correlation models. Such results represent a relevant proof of technical feasibility for the future clinical application of this motion compensation strategy in particle therapy.

INNOVATIVE METHOD FOR NON-INVASIVE PIVOT-SHIFT TEST QUANTIFICATION

Cecilia Signorelli - Supervisors: **Ferrigno Giancarlo**

Quantification of joint laxity is a critical issue in case of Anterior Cruciate Ligament (ACL) injury and surgery. In fact, ACL laxity measure serves in diagnosis, in evaluation of the severity of the ligament injury and also intraoperatively and postoperatively to quantify surgery outcome.

Clinical evaluation of ACL injury is performed by the execution of several clinical tests. Speaking of knee joint laxity it is important to discern static and dynamic laxity. Static laxity involves only one degree of freedom while dynamic laxity involves the whole joint kinematics and it is frequently presented as a symptom such as the feeling of "giving away".

The simplest tests that are able to assess knee static laxity are Lachman, Drawer and Varus-Valgus test. On the other hand, Pivot-Shift (PS) test highlights the dynamic behavior of the knee joint. Even if the static laxity tests are simple to perform and Lachman test is the most sensitive test in ACL diagnosis their outcomes result poorly correlate with symptoms, instability and patient's satisfaction.

Contrary to the static laxity test, PS test results to be correlated with joint instability, relief of symptoms, functional outcomes and patient's satisfaction after ACL reconstructive surgery.

Moreover it represents the most common symptom associated with ACL injury. Given that, the elimination of PS phenomenon is one of the main goals in ACL reconstruction. Currently, the ability to perform a correct diagnose of the injury severity as well as quantification of recovery after surgical treatment is mainly based on the surgeon's sensibility in interpreting the clinical examination. In fact, the main problem in the use of PS test is its complexity which makes itself a surgeon-subjective clinical examination.

To overcome this limit, during the last decades different kinds of arthrometers have been developed. However these tools are only able to measure the anterior-posterior laxity and not the dynamic rotation highlighted by the pivot-shift.

Different studies reported an intraoperative evaluation of dynamic laxity using Computer Assisted Surgery (CAS) system as they allow a quantitative evaluation of PS test. Unfortunately being highly invasive a navigation system results applicable only during the surgery and excludes the possibility to evaluate the contralateral limb. Anyway, the proved reproducibility and accuracy of the CAS system for ACL laxity evaluation support their use as the reference gold standard against which other

devices should be tested.

The present thesis presents a simple and non-invasive method for knee dynamic laxity assessment. The identification of the proper device, the definition of the laxity parameters, the determination of the protocol procedure for laxity assessment, the software development as well as the validation of the purposed method are all issues analyzed over the course of the present work.

Since the PS is a complex phenomenon that involves the whole joint kinematics, the idea is that a dynamics signal, such as the acceleration, can hold the information necessary to make a proper joint dynamic assessment. In order to be used during the clinical practice it is important that the purposed device results to be non-invasive, economically convenient, simple to be used and able to perform an automatic diagnosis with reproducible results.

The purposed device for dynamic laxity assessment consisted in a tri-axial accelerometer skin fixed to the lateral aspect of the tibia by an hypoallergenic strap. From the analysis of the clinical requirements and PS biomechanics, the technical specification of the device were chosen as following: 32 g as weight, (58 x 35 x 16) mm as dimensions, acceleration range ± 6 g, resolution 3 mg (± 6 g

Range), sample rate: 100 Hz, temperature range -40 °C / + 85 °C, Power Li-Ion Battery 5.0 V (rechargable).

The accelerometer was wireless connected to a tablet computer, provided by a specifically designed software, that was then commercially developed. The designed software specifically gave the possibility of automatically detecting the PS signal and extracting the defined laxity parameters. Given that, it allows the automatic quantification of the PS phenomenon analyzing the recorded signal while executing PS-test itself. In details, a signal template, which reproduced the 3D acceleration average trend while PS phenomenon occurs, was passed along the whole signal in order to recognize the presence of similar patterns. The acceleration signal was sampled at 100Hz. The recognition of the signal interesting share was based on the calculation of the Pearson's correlation coefficient between the template and the corresponding part of the windowed signal. The data acquisition concerning the 35 patients was used to define the template. The followed methodology has assured a recognition of PS repetitions with an accuracy of 96.7%, a sensitivity of 81.7% and a specificity of 99.3%. The results confirmed that it can be considered as a valid method for the automatic screening of the acceleration signal during PS test.

Within the acceleration signal different parameters were identified as significant: the maximum, the minimum, the range and the averaged jerk between the maximum and

minimum. The proposed method was tested in different clinical studies, aimed to highlight: (i) the influence of the muscular contraction and guarding during the acquisition; (ii) the reliability of the defined parameters in detecting ACL lesion; (iii) the comparison with a navigation system used as a gold-standard for PS quantification. All the clinical evaluations were performed at Istituto Ortopedico Rizzoli (Bologna, Italy).

The anesthesia on the purposed method was also analyzed confirming that the there were not statistical significant differences between pre-to-post anesthesia for the defined laxity parameters.

The clinical validation was performed on 66 consecutive patients who underwent ACL reconstructive surgery. The method showed a good reliability as the mean intraclass correlation coefficient was equal to 0.79. Moreover, the ACL-deficient knees presented statistically higher values for the identified parameters than the controlateral healthy limbs. A validation of the purposed device has been performed using a commercially available navigation system. An expert surgeon intraoperatively executed the PS test on fifteen patients before ACL reconstruction surgery. The anteroposterior tibial acceleration calculated by the navigation system and the acceleration obtained by the non-invasive sensor were compared obtaining a good direct correlation between them (rs=0.72, P<0.05). Even the evaluation of the soft tissues artifacts was assessed underlining a RMS displacement

equal to (4.9±2.6) mm. The obtained results confirmed the feasibility of the purposed method in dynamic laxity assessment during the whole patient evaluation in clinical practice and operating room, as well. It is worth noting that the described method represent a simple solution in case of ACL injury which may help the surgeon in quantify the dynamic laxity without requiring excessive expenditure of costs and time. The non-invasiveness of the tool has the dual advantage of allowing both the comparison injured-to-contralateral joint as well as the applicability even in the private practice were the CAS system are useless.

The surgeons need and are ready to obtain quantitative evaluation about knee laxity and this approach results useful in customizing the surgical approach and treatment plans be patient specific. In conclusion the presented device represents a reliable, not-expensive, simple to use quantitative aid in the preoperative and non-invasive ACL injury evaluation as well as during the intraoperative dynamic laxity evaluation and also for the assessment of functional recovery during follow-up controls.

NEW METHODS FOR MONITORING LUNG FUNCTION IN PRETERM NEWBORNS TO OPTIMIZE VENTILATORY SUPPORT

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Preterm birth is a significant perinatal health problem across the globe, affecting approximately 9.6% of all births worldwide (12.9 million/year) (Beck et al. 2010). The preterm lung is structurally and biochemically immature and vulnerable to injury and this has a great impact in terms of associated mortality, short and long-term morbidity and financial implications for health-care systems (Petrou et al. 2011). Several studies suggested that combining less invasive care strategies that avoid excessive oxygen and ventilation may decrease the incidence and severity of chronic pathologies as bronchopulmonary dysplasia (BPD) (Jobe 2011). Customization and continuous optimization of the treatment from the first moment of life on play a key part to avoid secondary injure. In particular, it is important to: i) recruit the lung at birth without overdistending it, ii) keep it open by applying the lowest pressure that prevents derecruitment during invasive or non-invasive supports and iii) adjust the settings according to the lung function changes related to lung growth and to the course of the pathology. However adequate tools available at bedside that can monitor lung function to guide the physician in identifying optimal ventilatory settings

during resuscitation at birth as well as during invasive and non-invasive ventilation are still missing.
The aim of this work is to develop methods and technologies to monitor lung function at bedside during the different approaches of the ventilatory support in preterm newborns to provide tools that improve the tailoring of the ventilatory strategy in order to minimise the negative outcomes that permanently compromise the quality of the future life of these infants.

Invasive ventilation. We identified the measurement of input impedance (Zin) of the respiratory system by forced oscillation techniques (FOT) as a promising tool for monitoring lung function during invasive ventilation. In fact, with an appropriate set up Zin can be easily monitored during clinical practice and animal studies have shown that it can identify an optimal mechanical positive end expiratory pressure (PEEP) in animal models of respiratory distress syndrome (RDS). Therefore we developed and validated in vitro new set-ups and methods to permit Zin measurements in preterm newborns. In vivo measurements proved them to be able to provide accurate Zin measurement without

interfering with breathing. Moreover Zin was useful to study clinical interventions that could modify end-expiratory lung volume and its distribution like PEEP and prone positioning. In particular we observed that Zin changes vs PEEP in infants with RDS present a similar behaviour to animal models of RDS, suggesting that a PEEP optimized on Zin bases could attenuate signs of ventilator induced lung injury (Kostic et al. 2011). The trend of Zin with PEEP in patients with BPD suggests that the role of PEEP in this population could be different and related to maintained airways patency. Even if these results are based on a small population and need confirmation, they suggest that Zin could have a role in optimizing the settings also for this group of patients. Moreover, the measured changes in the response to PEEP of Zin during the first week of life emphasize the importance of continuous adaptation of the treatment to the respiratory system condition.

Non-invasive ventilation. Nowadays significant efforts are directed to the improvement and development of new modalities of non-invasive ventilation in order to overcome the limits of the present technology and to avoid intubation in the majority of the infants. However the

mechanisms of functioning of the new modalities are still unclear and tools to optimise the support are still missing. During these modalities the assessment of lung mechanics is becoming more difficult as the interfaces with infants are developed to reduce dead space and improve infants' comfort. Moreover a crucial role is played by the infant's spontaneous respiratory activity and it is more evident in respect to invasive ventilation that the mechanical condition of the lung is the results of the interaction between the respiratory support and control of breathing. Therefore not only lung mechanics but also other indices could be useful to guide pressure titration. We identified the variability analysis of breathing pattern as a potentially useful tool as it permits to obtain information about control of breathing as well as mechanical properties of the thoraco-pulmonary system. Between the different method to quantify variability, detrended fluctuation analysis (DFA) has the advantage of not requiring the stationarity of the data and of distinguishing between intrinsic fluctuations generated by a complex system and those caused by external stimuli acting on the system. In a group of preterm infants treated with nCPAP in their first day of life, we found that optimising

nCPAP on oxygenation bases results into a lower long term correlation of EELV with respect to the absence of nCPAP or a high nCPAP value. These preliminary results suggest that variability analysis of the breathing pattern could provide useful information for tailoring ventilatory support. Studying the variability on different time scale of different signals that can be easily obtained in clinical practice and quantified it by different metrics is a possibility worthy to explore.

Resuscitation at birth. Finally, we applied Zin by FOT to monitor lung mechanics during resuscitation in order to provide important information for the optimization of this procedure. A dedicated set up has been developed to allow the measurement in such a delicate moment without the presence of technical staff. In collaboration with Acutronic Medical System (Switzerland) we developed and validated a set up that exploits FabianHFOV device for FOT measurements. Preliminary in vivo studies suggest that Zin can be useful to understand changes in respiratory mechanics due to this manoeuvre and to design a strategy of resuscitation at birth that maximises the final recruitment and minimizes the stress applied to the tissue during the manoeuvre. Having

this measure it will not be necessary to identify a procedure that works for all infants, but it will be possible to personalise the settings in real time based on the response of the respiratory system.

In conclusion, we developed new methods to monitoring lung function in preterm newborns. Even if further studies are needed to investigate the role of these measurements in the management of preterm infants, the performed in vivo studies have shown that they have the potential to be used in clinical practice providing important feedback on lung function to the clinicians.

PERCEPTUAL-MOTOR INTEGRATION AND MOTOR CONTROL IN DOWN SYNDROME

Sara Laura Vimercati - Supervisor: **Prof.ssa Manuela Galli**

Introduction

Down Syndrome (DS) implies a number of medical and health related complications, among which are cognitive deficits and neuropathology. While at birth it is often difficult to differentiate the brains of normally developing infants and of infants with DS rather clear differences between these two groups are present as early as 6 months of age. The development of motor milestones is achieved with consistent delay and cognitive developmental deficits lead to both mental retardation and to slowed and incomplete mastery of physical coordination. Three factors are traditionally believed to have an impact on the gross motor development of a child with DS: hypotonia, ligament laxity and decreased strength. Although these are well known features of DS, some authors have recently questioned their major role in causing the deficiency in motor skills acquisition. However, rehabilitation in DS is still based on generic strengthening therapies for the correction of these aspects. The lack of incisive therapies and focused rehabilitative guidelines for persons with DS puts forward the need for a deeper understanding of how the nervous system of these persons works and of how to help maximize their residual motor capacities.

Several studies have shown that the movements of individuals with DS are slower, less smooth and more variable when compared with the overall population. However, while specific sensory, motor, cognitive and perceptual impairments have been widely reported in DS, the way these localized deficits impact on perceptual-motor processing and function remains unclear.

In this study quantitative motion analysis is used to gain deeper insight on the major movement deficits caused by DS and to provide useful guidelines for a more focused rehabilitative treatment. The aim is to define and apply some experimental set up for the quantitative evaluation of the different aspects of motor control in adult subjects with DS:

The 1st part of the study analyses perception and extraction of sensory information, motor programming and decision-making processes during complex functional tasks in adult subjects with DS. Functional movements are based on real-world situational biomechanics and exert the interplay of high-level feedforward and feedback mechanisms;

The 2nd part of the study analyses simple, primitive mechanisms for motor control

in adult subjects with DS: the monosynaptic reflex and the pre-programmed reactions (PPRs). These mechanisms provide resistance to an external perturbation, are present since the very early stages of life and are very important for the acquisition of early motor skills. These experimental set ups were defined to have a general view of the motor difficulties in DS, from more primitive, automatic reactions to higher level mechanisms of motor control. **The novelty and scientific importance of this study is to provide insight on the mechanisms that regulate motor control in DS and to link these results to the most recent literature about cognition and neurology, to provide guidelines for a focused rehabilitation of persons with DS.**

Methods

37 adult subjects with DS and 43 controls participated to the study. This section is divided for the three movements evaluated, the first two belonging to functional movements and the third belonging to primitive mechanisms for motor control.
Obstacle avoidance during walking: the subjects walked in three conditions: plain walking without obstacle, walking with ground-level obstacle, walking with a higher obstacle

Arm tapping with and without obstacle avoidance: participants sat on a chair, holding a dowel with the dominant hand. In front of them was a table with six target positions evenly spaced around a semicircle. In the first condition (plain tapping) subjects tapped consecutively between targets. In the second condition (tapping with obstacle), subjects tapped consecutively and avoided an obstacle placed between two targets.

The tasks were acquired using quantitative movement analysis. Parameters were defined to analyze aspects of motor control such as: coordination of the limb segments, variability due to different conditions, sensory-motor integration, clearing strategies and trajectories, planning of the movement.
Analysis of monosynaptic reflexes and PPRs
The subject seated on a chair with his dominant arm flexed at approximately 90°, holding the handle of a bucket his hand. Two EMG sensors recorded the biceps and triceps activity of the arm. Subjects were told that the examiner was going to throw a weight inside the bucket and that they had to resist the perturbation and come back to the start position as soon as possible. A pressure switch recorded the instant of perturbation onset. The task was repeated 20 times. After signal processing the latencies for the monosynaptic reflex and for the PPRs were computed. The cross-correlation functions (CCs) between the pairs of averaged EMG signals were calculated to analyze the presence of synergies between the biceps and triceps muscles.

Results

The results from the first two experiments confirmed the picture of clumsiness and motor difficulties commonly described in DS. Moreover, they revealed that whereas the persons with DS seemed to correctly extract the sensory information (i.e. the characteristics of the obstacles) this information was not used in a pre-programmed fashion to plan the movement in advance, and thus their movement was highly dependent on feedback mechanisms, whereas controls relied more on feedforward mechanisms. The lack of anticipation in the planning of movement led to substantial modification of the movement parameters as the subjects approached the obstacle.
The parameters evidenced the presence of different movement strategies and the major effect of a disturbing factor, such as an obstacle, on the motor performance and quality of movements in DS respect to controls. The results demonstrated the presence of differences in high-level motor control of subjects with DS.
After shedding light on motor control in complex, functional situations, the third experiment addressed the function of primitive mechanisms for motor control to clear out if the differences in motor control that are observed in adults are present since birth or are a consequence of neural development. The results showed no differences between groups in terms of latency of the monosynaptic reflex and PPRs. Since these mechanisms develop in the early phases of life, these results provide evidence to the hypothesis that neuromotor

patterns in DS are comparable to those of normally developing infants at the very beginning of their sensorial exploration of the external world.

Conclusions

The finding that subjects with DS have comparable primitive mechanisms for motor control but employ different motor strategies in tasks that require higher control mechanisms seems to suggest that at birth there could be ample room for intervention.
Neural imaging studies about adults with DS support the hypothesis that a different shaping of the neural circuits occurs since the early phases of life in children with DS.
Given the high neural plasticity of the brain at birth and given the activity-dependent nature of neural modeling two most important suggestion can be given for rehabilitation:
Early intervention is fundamental for the reorganization of the neural pathways, and should be given as soon as the child is born.
Challenging the infant with an "enriched environment" may help balancing his lack of sensorial, cognitive and motor experiences, leading to earlier development of the motor milestones.
The future developments of this study should be addressed at correlating the kinematics and motor performance of individuals with DS with the underlying brain dynamics to better characterize the nature of the "clumsy" motor behavior observed in this population. This may be of fundamental importance in the definition of focused therapies.