MECHANICAL ENGINEERING | PHYSICS | PRESERVATION OF THE ARCHITECTURAL HERITAGE | SPATIAL PLANNING AND URBAN DEVELOPMENT | STRUCTURAL, SEISMIC AND GEOTECHNICAL ENGINEERING | URBAN PLANNING, DESIGN AND POLICY | AEROSPACE ENGINEERING | ARCHITECTURAL COMPOSITION | ARCHITECTURE, BUILT ENVIRONMENT AND CONSTRUCTION ENGINEERING | ARCHITECTURE, URBAN DESIGN, CONSERVATION OF HOUSING AND LANDSCAPE | ARCHITECTURAL, URBAN AND INTERIOR DESIGN | **BIOENGINEERING** | DESIGN | ELECTRICAL ENGINEERING | ENERGY AND NUCLEAR SCIENCE AND TECHNOLOGY | ENVIRONMENTAL AND INFRASTRUCTURE ENGINEERING | INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING | INFORMATION TECHNOLOGY | INTERIOR ARCHITECTURE AND EXHIBITION DESIGN | MANAGEMENT ENGINEERING | MATERIALS ENGINEERING | MATHEMATICAL MODELS AND METHODS IN ENGINEERING

PhD Yearbook | 2018

DOCTORAL PROGRAM IN BIOENGINEERING

Chair:
Prof. Andrea Aliverti

The main objective of the PhD Programme in Bioengineering is to prepare the PhD candidates to develop high level engineering problem-solving abilities in biomedical, healthcare and life sciences, inside research groups or in private/public industrial contexts, through a strong interdisciplinary training bridging engineering and medical/biological knowledge.
During the PhD, the candidates develop a scientific research project dealing with a complex problem which can be at different scales, from the molecular and the cellular levels to living organisms up to biomedical systems. They investigate original methods, devices, and systems with different purposes: increasing knowledge, proposing innovative methods for diagnosis and therapy as well as improving healthcare and daily life structures and services. At the end of the PhD programme, the candidate are expected to be able to carry out innovative projects and research and development in the field of Bioengineering, by proposing new methodological and technological solutions and properly evaluating the technology impact in healthcare, life science and biomedical industry.
During the three years of the program, PhD candidates perform their research through theoretical and experimental activities in four major areas: biomimetic engineering and micro-nano technologies; rehabilitation engineering and technology; technologies for therapy; physiological modelling and non-invasive diagnostics.
More specific areas include but are not limited to: molecular and cellular engineering, biomaterials, tissue engineering, bio-artificial interfaces and devices, neuro-prostheses, movement analysis, cardiovascular and respiratory system bioengineering, central nervous system signal and image processing for rehabilitation, biomechanics, computational fluid-dynamics, computer assisted surgery and radiotherapy, robotics, artificial organs, implantable devices, biomedical signal and image processing, E-Health, bioinformatics, functional genomics and molecular medicine.
The PhD Program in Bioengineering is organized with an inter-departmental structure. Faculty members of the PhD Advisory Board belong to two Departments of the Politecnico di Milano, namely DEIB (Department of Electronics, Information and Bioengineering) and CMIC (Department of Chemistry, Materials and Chemical Engineering "G. Natta").
PhD candidates (who are, in average, 20 per year) develop their PhD research programs within experimental laboratories located at the Politecnico di Milano or outside it, typically biomedical research centers, hospitals or industries.
When the research is performed within the Politecnico, PhD candidates are usually assigned to one of the following laboratories belonging to the DEIB and CMIC: Laboratory of Biological Structure Mechanics (LaBS, CMIC), Laboratory of movement analysis "Luigi Divieti" (DEIB), Medical Informatics laboratory (DEIB), Neuroengineering and medical robotics Laboratory (NearLab, DEIB), Biosignals, Bioimaging and Bioinformatics Lab (B3 lab, DEIB), Biomaterials laboratory (CMIC), Biomedical Technology Lab (TBMLab, DEIB), Experimental Micro and Biofluid dynamics (μBS Lab, DEIB), Computational Biomechanics Lab (DEIB), Biocompatibility and Cell culture Lab (BioCell, CMIC), Bioreactors Laboratory (CMIC). The Istituto di Elettronica, Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT) of the Consiglio Nazionale delle Ricerche (CNR), which is located at DEIB, represents another possible option.
Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the PhD candidate training.
The candidates are encouraged to carry out part of their research activities in contact with other research groups, preferably abroad through periods of at least three months spent in laboratories where the candidate can acquire further skills to develop his/her research work and thesis.
Collaborations that may involve the PhD students are presently active with several national and international research and academic Institutions. Very often, the involvement of companies and clinical partners facilitates the technological transfer of applied research into industry and clinical applications.
The educational offer includes *ad hoc* advanced courses specifically designed for the PhD in Bioengineering. The offer includes also the school of the National Bioengineering Group, which is held yearly for one week in Bressanone (Bz). Every year, the School is focused on different topics. As examples, the themes of the last few years have been: Neuro-informatics (2011), Biomedical devices from research to market (2012), Regenerative medicine (2013), From functional recovery to artificial organs (2014), Experimental models for development methods for 3R (2015), Bioengineering for Active ageing (2016), E-Health and digital medicine (2017).
The PhD Board of professors ('PhD Board') is composed by highly qualified and active researchers in Bioengineering, belonging to DEIB and CMIC. The PhD Board is responsible of all the candidate's activities. The competencies of Faculty members cover a wide spectrum of research fields. This allows a continuous updating of the PhD program and ensures that the PhD candidates are involved in innovative work.

COMPOSITION OF THE PHD BOARD

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

BIOMECHANICAL IMPLANTABLE DEVICE MODELLING: CRITICAL ISSUES FOR RELIABLE PREDICTION OF FATIGUE BEHAVIOUR

Dario Allegretti - Supervisor: Giancarlo Pennati

The design of implantable biomechanical devices is aimed at obtaining an effective performance and at gaining long-term reliability. Indeed, many of these devices are subjected to a high number of cyclic loads and are still affected by fatigue failure. A detailed approach to the durability analysis for biomechanical implantable devices is depicted from inputs based on clinical data, in vitro measurements and numerical simulations. Certainly, an important point is the definition of the in vivo loading conditions, which derive from the interaction between the device and the diseased area after implantation. Generally, literature studies recognize most of the difficulties in modelling the diseased patient area, while considering more straightforward the definition of the device numerical model. Nevertheless, some issues can also occur in the device modelling that, when not properly addressed, make the fatigue assessment unreliable. For this reason, the PhD thesis focuses on the device numerical description and the approach to evaluate fatigue endurance, highlighting various non-trivial aspects to be considered. Two biomechanical implantable devices are studied: the NiTi peripheral stents and the Titanium spinal rods.

NiTi stents were studied to assess the influence of a complex geometry in the device modelling. Moreover, the NiTi material description was performed by means of different approaches: an evaluation of material specimens, when available, and an innovative identification method applied directly on the stent. The identification approach exploits the surrogate-assisted optimization and resulted to give positive results in the improvement of the fatigue assessment reliability. Lastly, the standard approach for NiTi stent durability assessment requires the comparison between the actual stress-strain field in the device, regarding strain, and a reference material strain limit. Since the strain field induced into the stent is multiaxial and non-proportional, the comparison with a material limit requires the adoption of a criterion. Several literature fatigue models for metals were evaluated in the prediction of the fatigue behaviour of the NiTi stents since none of them is particularly formulated for shape memory alloys. In Figure 2 is reported the prediction of one of the fatigue criteria studied (Fatemi-Socie model) when applied on a stent under cyclic loading condition. The criterion was able to correctly predict the occurrence and the location of a fatigue fracture.

Titanium spinal rods are contoured to fit the spinal curvature before implantation. This characteristic permitted to study the influence of internal residual stresses in the device fatigue behaviour. The outcomes evidence a strong influence of the residual state of stresses after preimplantation procedure and highlight how this issues could both increase or decrease the device long-term performances. In Figure 3 are reported the results of the cyclic test performed on contoured bar in kyphotic and lordotic configuration. The outcomes show the influence of the residual stresses on the fatigue life.

The diversity of the two devices permitted to illustrate wide aspects of the modelling and to derive some conclusions which can be extended to other biomechanical applications. An adequate number of samples, available for both the devices, was used in several experimental investigations to validate the numerical findings. In conclusion, the thesis presents several outlooks. In the field of the academic and clinical research, it gives an upgrade in the state of the art and allows to improve the reliability in preoperative planning and postoperative follow-up. In the industrial developments, it could give high improvements in the design of the devices and could permit to work with a focus on the optimization of the long-term performance of biomechanical implantable devices, trying to set the bar higher in the field.

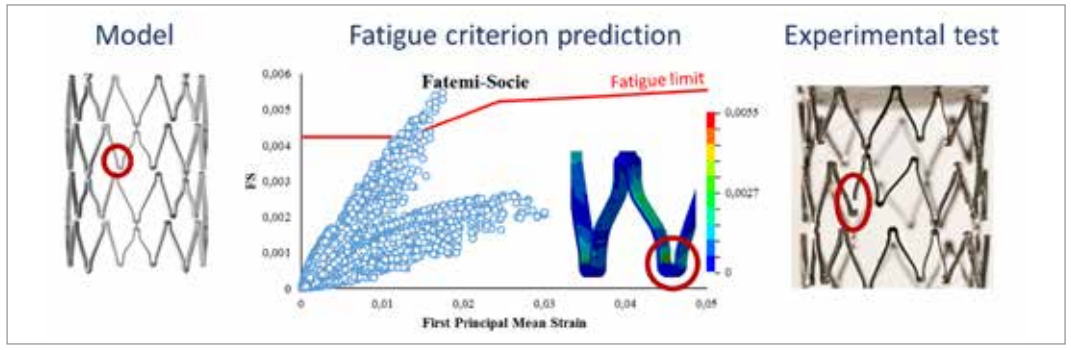

Fig. 2 - Prediction of one of the fatigue criteria studied (Fatemi-Socie model) when applied on a stent model in a finite element analysis under axial cyclic loading condition. The criterion was able to correctly predict the occurrence and the location of a fatigue fracture when compared with an experimental test.

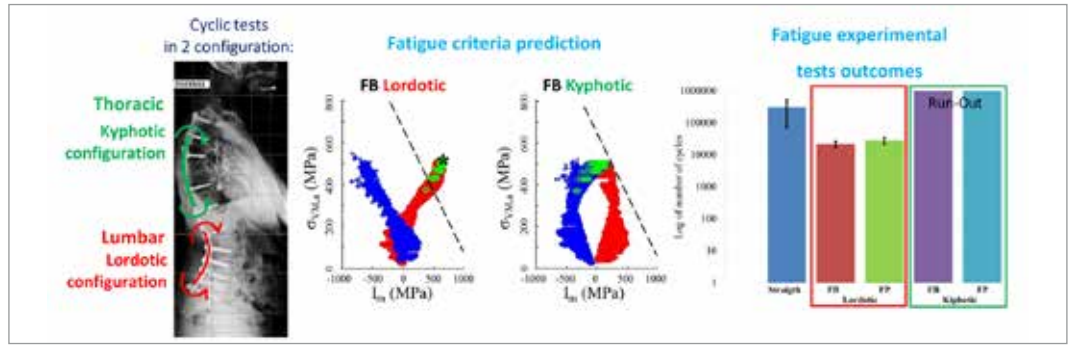

Fig. 2 - Results of the cyclic test performed on contoured bar in kyphotic and lordotic configuration. The outcomes show the influence of the residual stresses on the fatigue life.

MATHEMATICAL TUMOR MODELLING TOWARD A PERSONALIZED APPROACH IN RADIATION THERAPY.

Antonella Belfatto - Supervisor: Prof. Pietro Cerveri

Treatment personalization in clinical oncology addresses the problem of large variability in the patient response to radiotherapy. Despite the amount of possible therapeutic options, treatments still stem from general guidelines: once the stage of the disease is assessed, the therapy is conveyed to the patients in an almost one-size-fits-all fashion, according to the facility protocols. In this clinical context, mathematical models have been introduced both to provide insight about the biological mechanisms and to predict the patient prognosis. It has emerged from clinical studies that simple radio-biologic models, such as the linear-quadratic model, failing to incorporate the radio-sensitivity heterogeneity and/or tumor cell repopulation, cannot adequately describe clinical outcomes. This implies that a complex network of interdependent dynamics drives the overall tumor progression.
First, it was observed that while part of the tumor consists of viable cells, which either keeps dividing or rests in a quiescent state, another portion might be necrotic due to either lack of nutrients or treatment effects. Moreover, micro-environmental factors (e.g. oxygenation), affect the tumor aggressiveness and responsiveness to the treatment.
In the last decade, many attempts in defining more accurate and reliable mathematical models have been made. All the works developed in the field of tumor growth modeling can be coarsely classified into two different groups: macroscopic and multiscale models. On one hand, macroscopic models focus their attention on the tissue level and are able to provide a big picture of the pathology evolution. On the other hand, multiscale models allow the interplay among the overall tumor evolution and the mechanisms occurring at genetic/cellular level. However, their reliable and accurate setting requires a multimodal data gathering which may include invasive and expensive methods. As a consequence, the advantages of the multiscale architecture could be hindered in clinical practice. Conversely, macroscale models, despite the approximation of the complex phenomena underpinning the tumor evolution, can provide useful information on the tumor evolution while requiring a reduced data gathering effort which is feasible and even already performed, in everyday clinical practice. In the light of these premises, we propose macroscopic models defined by means of equations systems describing the tumor volume/mass change across time. Starting from two-dynamics (viable and necrotic portion) systems, other mechanisms such as the effect of the oxygenation or the occurrence of edema, are introduced according to specific applications. Models are trained and tested on different data types of prostate bearing rats and cervical cancer patients. We also suggest their possible inclusion within a treatment planning scheme. A tumor model is built to represent the evolution of a general solid tumor. It is specialized by training the parameters on a dataset of a specific tumor type (group-specific model). When a new patient has to be treated, different fractionation modalities are simulated. The clinician, based on its expertise, the institutional guidelines and the model simulation, selects the treatment strategy. The tumor evolution is monitored and the new data are used for a model parameter refinement. New simulations are performed and the treatment strategy may be revised accordingly.
First, we investigated the fitting ability of the two-dynamics system on the tumor evolution of prostate cancer bearing rats. Among the 18 rats included in the study 9 were breathing oxygen while 9 inhaled air during the irradiation. Caliper based measurements of the volume evolution along with magnetic resonance acquisition providing oxygen information were available. Despite the limitation of a small dataset, the caliper-based measurements and model dynamics reduction, the proposed formulation was able to fit the data within about 25% error in 15 of 18 rats. The correlation analysis suggested a relation between the radio-sensitivity and the changes a MRI-based index for a subgroup group. This hypothesis was supported by further investigation leading to the finding that only rats featuring an increase in the same index during an oxygen challenge, seemed to benefit from oxygen inhalation.
In the end, we showed how the radiosensitivity could be assessed *a priori* using a neural network by means of oxygen and volume-related information.
Afterwards, we introduced the hypothesis that the larger the active portion the higher the oxygen consumption and the lower the average oxygen availability. We also assumed that an increase in oxygenation results in an improved radiosensitivity and that the irradiation damages to the microenvironment can affect the tumor regrowth. In one study, 7 cervical cancer patient were included, and both volumetric and vascularization/flow information were available for validation due to US-Doppler imaging. The high correlation value (r~0.9) found between the average oxygenation predicted by the model and the corresponding tumor reduction (inter-patient) supports the hypothesis that the oxygenation is a prognostic factor of the tumor response. We also found correlations (r>0.5 up to r=0.95) between the model-based oxygenation and the Doppler indices in 5 out of 7 cases. Poor correlation only occurred in two patients showing a very hypoxic condition recognized by the model, suggesting that in case of severe hypoxia recognizing its occurrence can be more relevant than predicting the oxygenation trend.
We also addressed the possibility to adjust the model parameter starting from a group-based setting to reach a patient based configuration by exploiting a two-step procedure of parameter optimization. This study was carried out on 16 cervical cancer patients. The parameter adaptation of the group-based model reduced the prediction error of about one half with respect to its initial value (error range: 20-15%) reaching promising performances (error range: 7-10%). These results, comparable to the fitting performances (mean error: 5%) of the patient-specific parameter optimization investigated in a previous study, suggested that this novel approach can successfully customize the group-specific model. Moreover, a similar error range was found when the adaptation was performed using the data of the first two week of treatment only. In other words, exploiting two weeks of data the model is able to remarkably improve its ability to predict the tumor evolution on a patient-specific basis.
Finally, we investigated the model ability to cope with different treatment strategies by means of simulations based on one of the above mentioned models. We found that treatment pauses as well as different fractionation strategies affected the model simulation and discussed on the possibility of defining a ranking system to select the best treatment for the specific patient according to the simulation results, for example considering the maximum volume reduction. Despite the preliminary nature of the project, which should be addressed including larger dataset, the results are promising. The studies present many novelty aspects including the simplified dissertation of complex phenomena within a simplified mathematical tool, the multimodality data validation and the idea of an online model tuning to move from a general setting to a patient specific configuration. We addressed the modeling problem from several angles and provided interesting hints for a clinical-oriented application. Therefore, we believe that the work described represents a step forward in the radiotherapy personalization for oncological patients.

PATIENT-SPECIFIC MODELLING FOR HEMODIALYSIS THERAPY SETTING OPTIMIZATION

Camilla Bianchi - Supervisor: Prof. Maria Laura Costantino

Hemodialysis (HD) is the most common therapy to treat the wide range of patients suffering from end stage renal disease. In the most of the cases it is the only applicable solution to keep patients alive, even though the impact on the patient's cardio-vascular and hydro-electrolytic equilibrium is still nowadays of great relevance. It also has high socio-economic and environmental impact on health systems worldwide.
In the last 30 years many technological improvements have been introduced in the hemodialysis treatment process: from enhancing the biocompatibility and purification performances of the filter membrane to on-line treatment monitoring by using biofeedback sensors, passing through the introduction of high-efficiency treatments. What bioengineering can still do to improve patients' life quality and individual tolerance is working, in close cooperation with clinicians, on HD treatment customization. An example of these efforts is the *DialysIS* ("Dialysis therapy between Italy and Switzerland") Project, whose results satisfactorily achieve the goal of treatment tailoring by means of development and sharing of optimised clinical protocols and introduction of decision support tools to improve dialysis prescription. In the framework of the mentioned project, this doctoral thesis proposes a set of integrated mathematical tools to be introduced in clinical practice in order to better manage the patient-specific hydro-electrolytic condition and prevent intra-dialysis complications.
A multi-pool parametric kinetic model has been developed (see Fig. 1) and later validated, thanks to a great amount of clinical data acquired during *DialysIS* Project. It has been coupled with two different approach-based optimization algorithms, in order to properly identify the parameters defined as patient-specific in the mass and fluid transport phenomena involved in the HD process. The chosen optimization methods are a constrained non linear optimization method and a Bayesian estimation approach.
The results of the description and prediction capabilities evaluation of the model are very satisfactory. Indeed, the model shows a median error always lower than 4% when plasma electrolytes and catabolites and blood volume trends are described, while the median error is always lower than 6% when the same variables are predicted, with overall reduced interquartile ranges. Furthermore, the compatibility between the mathematical artifice in identifying the personalised parameters and the physical explanation of the problem has been thoroughly investigated, by means of ad-hoc performed multiple linear regression analysis. Results of this have shown correlations between kinetic parameters identified by the mathematical algorithm and patient's blood clinical measurements, confirming the expectations. In some other cases, it has been found that the parameter values identified are strongly affected by the mathematical constraints.
A brief focus on the most common intra-dialysis complication, namely intra-dialysis hypotension (IDH), has been carried out. The clinical applicability of a set of IDH definition criteria and of a multi-parametric algorithm to prevent IDH has been evaluated. These tools demonstrated to be reliable in managing IDH in advance, giving warnings that could reduce IDH onset of 54% (with a specificity of 99.6%), thanks to preventive, instead of corrective, clinical fundamental interventions.
Finally, an in-vitro simulation platform of the HD patient has been proposed to better investigate the patient-machine interaction. On one hand, the optimization and preliminary tests of a two-pool virtual patient simulator encourage its use to study the different patients' vascular compensations mechanisms in response to HD treatment. On the other hand, the in-vitro simulation platform has allowed to perform some preliminary tests to develop a protocol for dialyser membrane efficiency evaluation as a function of blood protein content. This protocol could be applied to a wider range of current dialyser tests, in order to study protein adhesion and consequences on the overall mass and fluid transport across the dialyser.
All these experimental tools could be then coupled with the mathematical model to achieve the best results from treatment customisation and improve HD therapy clinical prescription, as well as prevent HD related complications.
The primary aim of this doctoral thesis is the development of a patient-specific multi-compartment kinetic model for the application in clinical nephrology to achieve hemodialysis treatment customization. The experience and data collected while achieving this primary aim allowed to carry out an additional activity, related to experimental platforms to study the patient-machine interaction. Mathematical modelling indeed can be promising in order to predict the patient-specific response to the treatment. However, an in-vitro platform is necessary to complete the investigation of the correlations among variables involved in the complex integrated system represented by the patient and the HD machine. The aims of this work are related to the need for clinical support tools to improve hemodialysis prescription and setting. These tools have to be non-invasive for the patient and represent non-causative agents to the economic and environmental impact.
The natural kidney is capable of constantly maintaining complete homoeostasis with complex sensing and bio-feedback mechanisms to react upon changing circumstances. In contrast to the natural kidney, conventional HD instrumentation is based on an approach of replacing the functions of the damaged complex renal system through standard settings usually applied to the entire range of patients. This approach should be abandoned and hemodialysis should be prescribed on an individual basis instead of on a facility level. Kinetic modelling of body fluids and vital electrolytes concentrations during dialysis can enable such an individualized and tailored dialysis prescription, thereby preventing or at least mitigating complications. The doctoral thesis is focused on the identification of new mathematical tools and experimental methods to be developed in association with the clinicians to improve dialysis therapy outcomes.

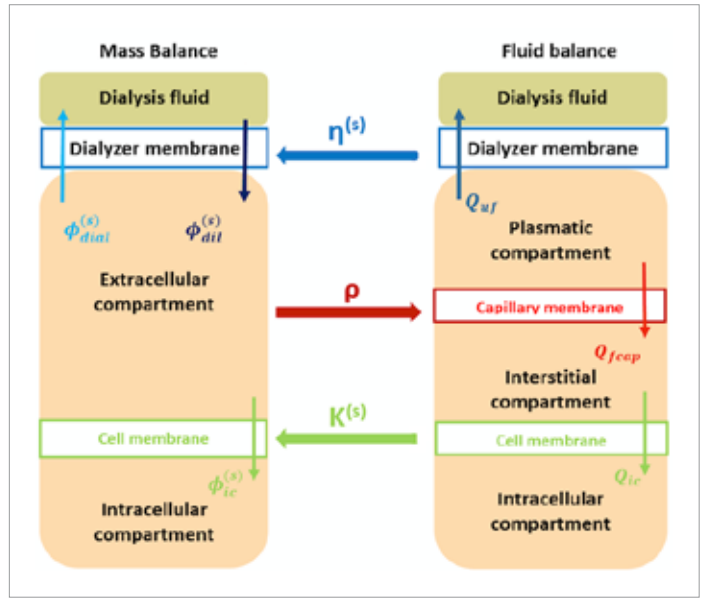

Fig.1 - Body compartments of a HD patient with fluid and mass exchanges across the biological and artificial membranes (patient-specific parameters ρ, $k^{(s)}$ and $\eta^{(s)}$ are highlighted where they act).

A NETWORK-GUIDED MODEL PREDICTS SENSITIVITY TO ALL-TRANS RETINOIC ACID IN A TUMOR-TYPE INDEPENDENT MANNER

Marco Bolis - Supervisor: Prof. Linda Pattini

All-trans retinoic acid (ATRA) is routinely used in the treatment of acute-promyelocytic-leukemia (APL), a subtype of acute-myeloid-leukemia (AML). In combination with chemotherapy or arsenic trioxide, ATRA induces complete and long-lasting remissions in the majority of APL patients. In this context, and unlike classic chemotherapeutics, ATRA exerts most of its anti-tumor activity by promoting cyto-differentiation rather than direct cytotoxicity. In vitro experiments suggest that ATRA may be effective in diseases other than APL, such as breast cancer and brain tumors. Nevertheless, there is no clear indication that can be used to identify potentially sensitive tumor subtypes and thus retinoic-acid is still under-exploited in other malignancies. The unusual mechanism of action, the clinical results obtained in APL and numerous pre-clinical data in other types of neoplastic disease have raised interest in ATRA. A rational use of ATRA in oncology calls for the identification of the types of neoplasia which are most responsive to the anti-tumor activity of this natural retinoid. In view of precision medicine approaches to treatment, it is also important to predict and confirm ATRA responses in single patients independently of the tumor-type. In the present study, I developed a gene-expression based model which is associated with and predicts ATRA-sensitivity in a tumor-type independent fashion. The approach used to define the model took advantage of the experimental data on ATRA sensitivity obtained by our laboratory on a large panel of breast cancer cell-lines and the corresponding basal gene-expression profiles. Selection and application of machine learning algorithms resulted in the identification of an original model of 139 genes (ATRA-139) capable of predicting ATRA sensitivity in our cell-line training- and test-sets with high confidence. Surprisingly and despite its original development in mammary tumors, when applied to a completely different tumor context such as leukemia, the model could discriminate ATRA-sensitive APL from other AML cases. This prompted us to further optimize the model. Using a network guided-approach, we investigated expression and interaction of ATRA-139 to reduce the number of features to those that were likely to be of relevance across different tumor types. The results obtained allowed us to develop a generalized model (ATRA-21) whose validity extends to tumor-types other than breast cancer. *ATRA-21* predictions correlate with experimentally determined sensitivity in a large panel of cell-lines representative of numerous tumor-types. In patients, *ATRA-21* correctly identifies APL as the most sensitive acute-myelogenous-leukemia subtype and indicates uveal melanoma as the neoplasia characterized by the highest predicted sensitivity, followed by low grade glioma, thyroid cancer, paraganglioma/ pheochromocytoma, diffuse large B-cell lymphoma and adrenocortical cancer. The results described above provide insights into the types of tumors that are likely to represent targets for the design of ATRA-based therapeutic strategies. To further validate the predictive potential of ATRA-21 we compared our sensitivity predictions in AML patients to the results obtained by a recent clinical trial performed with the addition of ATRA to the standard therapeutic protocols. We used ATRA-21 to determine sensitivity in 1289 acute myeloid leukemia (AML) patients from 4 publicly available datasets. Our predictions support the idea that other AML sub-types, besides the expected acute promyelocytic leukemia (APL) group, are likely to be responsive to the anti-proliferative effects of ATRA. Indeed, the ELN-favorable-risk, CEBPA double mutant, t(8,21) and inv(16) AML subgroups are characterized by high average ATRA-21 scores and are predicted to be sensitive to the anti-leukemic action of the retinoid. In a precision medicine perspective, ATRA-21, which consists of a restricted number of genes, is likely to represent a new tool for the selection of patients who may benefit from treatments based on the use of ATRA.

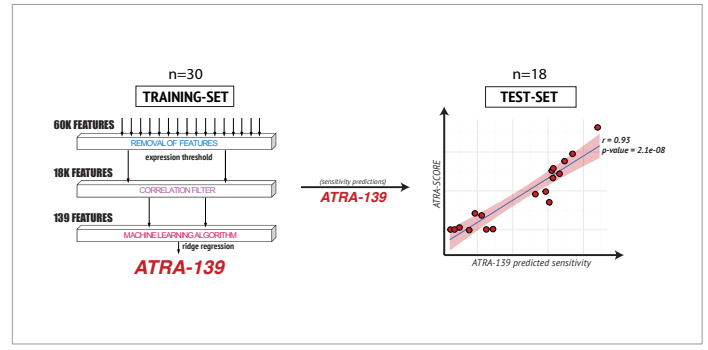

Fig. 1 - ATRA-139 model generation workflow _ Left: The workflow adopted in the selection of the features used for the training of the ridge regression model is shown. Total = 48 cell-lines; Training-set = 30 cell-lines; Right: The scatter plot shows the performance of the ATRA-139 model in predicting ATRA-sensitivity in the test set. Test-set = 18 cell-lines.

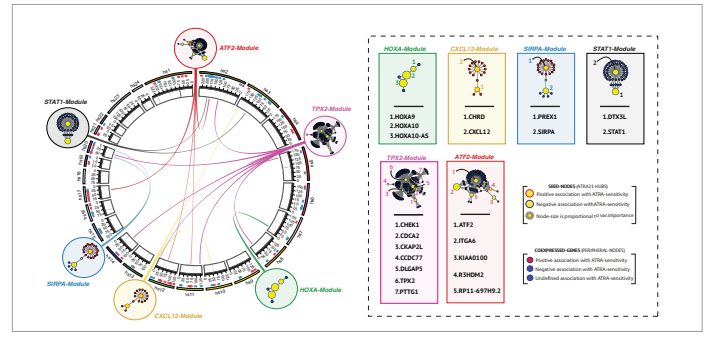

Fig. 2 - Consensus modules, ATRA-21 predictive model genes and genomic localization _ Left: The circos plot illustrates the genomic position of the ATRA-21 genes belonging to the 6 identified consensus co-expression modules. Right: The 6 identified modules (ATF2-, TPX2-, HOXA-, CXCL12, SIRPA- and STAT- Modules) and their component are show.

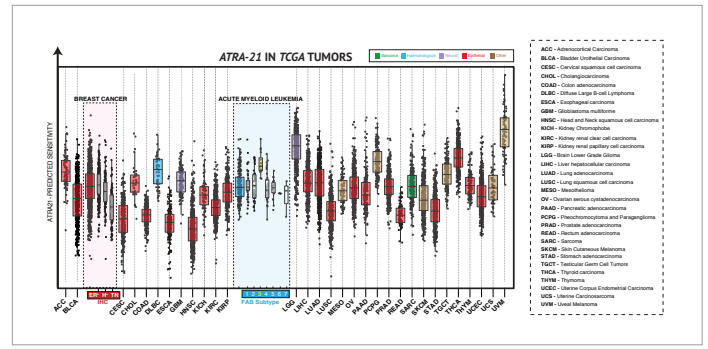

Figure 3 - ATRA-21 sensitivity predictions across tumor-types
The Box plots illustrate the distribution of ATRA-21 sensitivity predictions after grouping of the tumor-types (TCGA database). In the case of breast cancer and acute myeloid leukemias, cases are stratified according to immuno-histochemical subtype and FAB classification, respectively.

ENGINEERING VASCULAR TISSUE MODELS IN VITRO

Nina Bono - Supervisor: Prof. Gianfranco B. Fiore

In the last decades, vascular tissue engineering (vTE) has passed through the arduous path to successfully (re)create human blood vessels replacements for clinical therapies. However, in parallel to the quest for clinical applications, there is a side road, for now less traveled but still challenging, for engineered tissues that instead leads to the development of laboratory-oriented technologies to increase basic knowledge about the mechanisms involved in vascular physiology and pathophysiology and/or assessing drug response. In this context, tissue engineered vascular constructs (TEVCs) have proved able of providing *in vitro* models that are increasingly similar to native tissues, representing a powerful means to bridge the gap among *in vitro* results, animal models and human studies. Recent efforts have been focused on mechanotransduction as a key factor involving in vascular processes, including physiological functions and development of some pathologies (e.g. atherosclerosis). Therefore, through the development of 3D engineered models, not only it is possible to understand at a fundamental level how cells sense mechanical forces and transduce them into biochemical signaling, but also to identify the molecular mechanisms by which mechanical forces regulate vessel function. Since the control of cell phenotype and function is essential for the *in vitro* fabrication of engineered tissues with tailored properties, understanding how these changes occur in vascular physio-pathology is of great importance in the area of vTE.

In this context, the goal of this PhD thesis was to develop a suitable strategy to engineer 3D vascular tissues as *in vitro* models for mechanobiology investigations. The main line of the work was to capture *in vitro* some of the complex features of the *in vivo* vascular *milieu*, with particular focus on the replication of the vascular-like hemodynamics and the way how it influences cell and tissue behaviors. Since vascular smooth muscle cells (vSMCs) are the predominant cell type in blood vessel wall and primarily responsible for vasoregulation and vessel remodeling, major efforts have been devoted to the *in vitro* development of SMC-based engineered tissues as vascular models.

The focus of the 1st section of the work was to demonstrate that contractile vascular cells respond more physiologically in a 3D environment under cyclic mechanical stimulation. Specifically, a thorough comparison between 2D (cell monolayers) and 3D (cellularized collagen-based gels) models was envisioned with the aim to shed light and compare the effects of different cell culture configurations (i.e., dimensionality of the culture set-up) in combination with mechanical stimulation (i.e., uniaxial cyclic strain) on vSMCs behavior (Fig. 1). The study pointed out a substantial difference in response when cells are cultured in 2D or 3D environments, whether or not subjected to mechanical stimulation, thus pinpointing the importance of 3D dynamic cultures (i.e. a combination of environmental and mechanical cues) to study vSMCs, for gaining a control over vSMCs behavior *in vitro*, and in perspective to achieve functional SMC-based tissues. This study also confirmed the validity of collagen gel as a promising scaffold material for fabricating engineered vascular tissue models. On this ground, the studies described in the other two sections of this Thesis exploited tubular collagen gels populated with SMCs and subjected to different mechanical forces typical of the vascular hemodynamics, such as shear stress and cyclic strain. Besides, these two mechanical components are believed to contribute, *in vivo* as well as *in vitro*, to vascular remodeling. The main challenge to face was thus the development and stepwise optimization of a culture platform (i.e. a dual-mode bioreactor) for the fabrication of 3D tubular engineered tissues at once (Fig. 2). The role of flow-related shear stress and that of the circumferential cyclic strain on collagen-based constructs were studied independently. This was done with the aim to dissect the specific contribution of each stimulus on construct characteristics and on cells ability to reorganize their surrounding matrix, thus improving the overall mechanical stability of the constructs. Our results suggested that a dynamic culture period of about 1 week may be sufficient to observe and evaluate early mechanism involved in tissue remodeling, at least those involved in matrix compaction and cell alignment. The study have also shown novel and interesting results about the non-homogeneity of cell growth in static samples if compared to strained constructs. Indeed, static constructs exhibited an overall decrease in cell density, suggesting cell death occurred in deeper part of the constructs. It is noteworthy that, at least in the context of laboratory studies, the culture platform developed during this PhD is a valuable tool to create accessible, "off-the-shelves" 3D vascular tissues that, whether properly stimulated, can be tailored to specific applications. Additional investigations are, however, needed to elucidate the intimate relationships existing among cell alignment, the contractile phenotype of cells and the resulting mechanical strength of viable engineered tissues, thus providing new insights into mechanotransduction pathways activated after the exposure to mechanical strain in 3D environments.

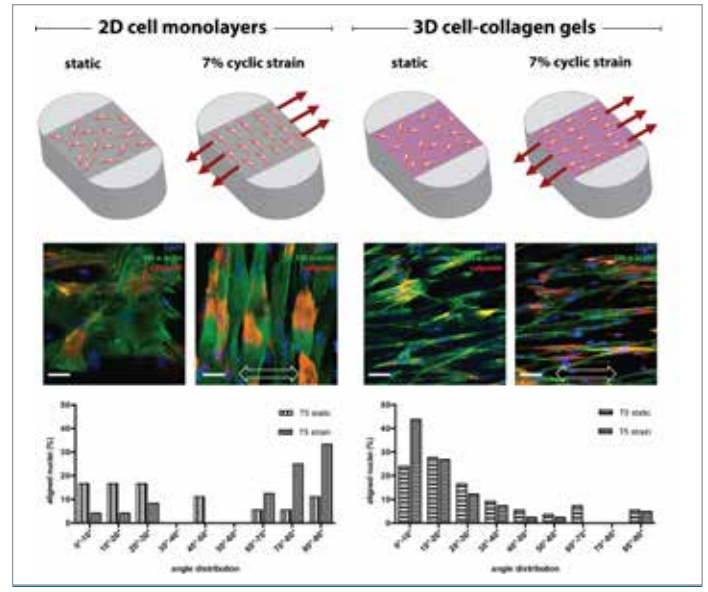

Fig 1 - Comparison between 2D (cell monolayers) and 3D (cellularized collagen-based gels) vascular models. Upper panel: experimental set-up; middle panel: IF images of cell alignment; lower panel: quantification of cell (nuclei) orientation.

In conclusion, in the whole context of the vTE, the general picture drawn in this PhD thesis highlights i) the importance of the 3D environment to study the cell behavior, and ii) the need of mechanically stimulating 3D vascular tissue structures to drive their biological and biomechanical properties and their maintenance *in vitro*. On the technological front, these studies have led to the development of a new easy-to-use and cost-effective bioreactor system that is very useful to produce viable and reproducible vascular models. In perspective, such constructs may be used to study the cell-cell and cell-extracellular matrix interactions occurring during vascular development in healthy and diseased conditions, as well as to dissect the multiple signaling pathways and the related transcription factors mediating the responses of vascular cells to changes in flow and stretch.

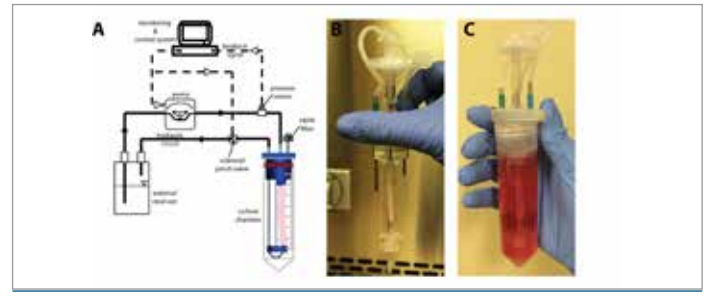

Fig. 2 - A) Layout of the dual-mode bioreactor. B-C) 3D (tubular) vascular models during dynamic culture.

CHARACTERIZATION OF METABOLOMIC SIGNATURES IN SEPTIC SHOCK PATIENTS: A DATA MINING APPROACH

Alice Cambiaghi

Supervisors: Manuela Ferrario, Giuseppe Baselli, Roberta Pastorelli

According to reports in the United States and in Europe, shock affects about one third of patients in Intensive Care Unit (ICU) for a total of more than 1 million victims a year. Septic shock is defined as a complication of sepsis characterized by a life-threatening organ dysfunction caused by a dysregulated host response to infection, which involves circulatory, cellular and metabolic abnormalities. This results in an overwhelming and uncontrolled inflammation with resultant impairment of the coagulation cascade and poor tissue perfusion. Without adequate oxygen delivery, tissues deteriorate and, consequently, organs begin to fail. This condition is known as multiple organ failure (MOF), i.e. organs not directly injured by infection become dysfunctional due to systemic disorders involving immunoregulation and endothelial dysfunction. When MOF occurs, the damage to tissues is already so extensive that the patient is destined to die, even with an adequate medical intervention. Current treatments for septic shock are mainly devoted to restore homeostasis and to prevent MOF. Since the transition to serious illness occurs in few critical hours, it has been speculated that early recognition and treatment administration could provide maximal benefit. The complex pathophysiology of sepsis suggests that a single biomarker approach cannot adequately describe this syndrome, thereby a comprehensive and integrated analysis of molecular and clinical measurements is needed to plan an early and appropriate therapeutic intervention.
In this context, information at molecular or cellular level provided by omics analyses (i.e. genomics, transcriptomics, proteomics and metabolomics) are a suitable approach to follow responsiveness to therapy and to establish new therapeutic targets. Metabolomics consists in the analysis and quantification of thousands of metabolites, i.e. small molecular compounds which constitute the end products of cellular metabolism and can thus be considered the chemical fingerprint of an organism at a precise time point. Metabolomics approaches have become a powerful tool for revealing molecular pathways and for identifying and quantifying differentially expressed metabolites, independently from multiple trigger factors causing the disease. This aspect is very promising for a complex and multifactorial syndrome as septic shock, and makes metabolomics analyses a suitable starting point toward personalized medicine.
Omics datasets are highly complex and they are characterized by a large number of highly correlated features (hundreds or thousands) compared to the number of observations. For this reason, traditional statistical tests cannot be used alone for a robust analysis but it is necessary to develop suitable strategies by combining different data mining techniques, studied ad hoc for the specific scientific question.
The aim of this study is to provide a thorough description of putative biological pathways, which characterize severe septic shock patients on a short temporal window in order to suggest possible biomarkers to be validated in further investigations.
We focused on a homogeneous and well defined group of patients, including two cohorts: the first cohort is constituted by a subset of 20 patients from ALBIOS database (NCT00707122), the second cohort of patients consists of 21 septic shock patients enrolled in ShockOmics clinical trial (NCT02141607).
The analytical approach proposed combines features reduction techniques, linear and logistic regression models and linear methods for classification in order to extract previously unsuspected information from omics datasets, which could identify the metabolic pathways mostly associate to the outcome under study. Briefly, to perform feature reduction we adopted the minimal-redundancy-maximal-relevance (mRMR) method proposed by Peng et al (IEEE Trans Pattern Anal Mach Intell. 2005;27(8):1226-38), which ranks the features according to their relevance to the outcome and to their redundancy with respect to the other variables. Successively, the featured selected and reduced in number were used to build logistic regression models with the elastic net regularization approach. The elastic net performs both variable selection and regularization in order to enhance the prediction accuracy and interpretability of the statistical model it produces. Linear methods for classification, i.e. Linear Discriminant Analysis (LDA) and Partial Least Squares Discriminant Analysis (PLS-DA), were also applied on our datasets.
In particular, two studies were performed on ALBIOS dataset. The first one is an explorative analysis aiming at providing absolute quantitative information on changes in plasma metabolite levels measured one day (acute state, D1) and one week (steady state, D7) after development of severe septic shock, so to relate these changes with mortality at 28 days. The two time points were chosen to verify the hypothesis that the metabolic changes over the time reflect not only initial clinical characteristics, but also the progression of the disease and the long-term survival.
Overall, our results showed that the metabolite species mainly involved are the kynurenine and lysophosphatidylcholines (lysoPC), confirming what already reported in previous studies.
In light of these findings, we speculated that lipid homeostasis and tryptophan catabolism might influence mortality in septic shock. Therefore, we further investigated the changes that occurred in metabolites concentrations (expressed as ratio D7/D1) and we integrated this information with the variation in proteomics and clinical data. The results obtained confirmed that early changes in plasma levels of lipid species are altered in non survivors. As for proteins, the most important differences between the two groups are related to proteins belonging to inflammation and to the coagulation cascade, which are two of the most important pathways involved in septic shock progression. In respect to our previous analyses on metabolomic data only, the integration with proteomics seems to indicate the importance of the interaction between inflammation, coagulation and the complement system in sepsis, which is in line with recent findings.
The study performed on Shockomics database aimed to elucidate early metabolic signatures associated with the progression of septic shock and with responsiveness to therapy, assessed as change in SOFA score measured at admission (T1, acute phase within 10 hours from shock diagnosis) and 48 hours after T1 (T2, post-resuscitation). The multivariate models showed that lower variation in the concentration of plasmalogens and of fatty acids, in combination with a higher increment of alanine, were associated to non-responsiveness. Furthermore, alanine indicated a possible alteration in glucose-alanine cycle, which occurs in the liver thus providing a different picture on liver functionality than bilirubin, which is usually used in clinics.
In conclusion, our results demonstrate the feasibility and the robustness of the proposed approaches, in spite of the limited number of patients. In fact, the performances of our classification models are good and the results are consistent with the literature and with investigations on the identified pathways. To the best of our knowledge, no other scientific works have applied data mining technique to perform multilevel omics analyses with the aim to find association between plasma metabolome changes and mortality or responsiveness to therapy. Thus, our findings (Sci Rep. 2016 Feb 5;6:20391; Sci Rep. 2017 Aug 29;7(1):9748) represent a significant advance in the field and could be an important step toward the devise of a personalized therapy.

CONE BEAM CT AND PROTON CT FOR ADAPTIVE RADIO AND PROTON THERAPY

Francisco Roberto Cassetta Jr. - Supervisors: Guido Baroni, Marco Riboldi

Introduction: Continuous technological developments in radio and proton therapy create new and better solutions for cancer treatment. The introduction of new image-guidance and adaptive therapy methods requires extreme caution and extensive verification should be made, accounting for expected results, possible gains over induced damage and implementation challenges.

Methods: This work is focused on image-guidance methods in radio and proton therapy, with specific emphasis on anatomical changes on patients during treatment. Automated image registration methods have been implemented and validated, in order to show the full potential of CBCT image registration. Geometric and dosimetric verification has been carried out, thus checking the feasibility of treatment adaptation relying on automated image registration. In addition, proton CT is investigated and verified as a low-dose method for patient positioning in proton therapy.

Results: Deformable image registration (DIR) (figure 1) followed by dose recalculation (figure 2) was evaluated for prostate cancer patients. Variations in patient anatomy were estimated (distances measured between landmarks) and corrected with DIR, resulting in a mean landmarks distance reduction from 3,79 to 2,00 mm. In 8 out of 9 patients an increase in rectum dose was observed, where, for 3 of them, important constraints of dose/volume were violated, which could result in acute reaction on patients' organs at risk. The proposed automatic contour propagation method by means of DIR was tested in rectal cancer patients, treated with proton therapy, where repeated in-room CTs were available. The DIR validations for these patients were carried out relying also on dose comparison. The gamma test presented an improvement on the pass fraction, on the most critical case, from 88,40% to 99,87%. The automatically generated contours required only few corrections which, nevertheless, would have small influence on final DVH for online analysis. The implementation of adaptive strategies would collaborate for healthy organs sparing and PTV coverage verification. Low-dose positioning methods with proton CT images (figure 3) were evaluated and a reduction of up to 55 times in comparison to a conventional CBCT was obtained. Proton CT images were demonstrated to be suitable for image registration at sub-millimeter confidence in patient positioning.

Discussion: Image registration algorithms based on open-source libraries were developed and tested for the realization of adaptive therapy strategies. The proposed methods facilitate daily dose calculation and plan verification (online or offline), thus providing higher precision and effectiveness of the treatment.

DESIGN, MANUFACTURING AND IN-VITRO TESTING OF AN INNOVATIVE BIOMORPHIC HEART VALVE MADE OF A NEW THERMOPLASTIC ELASTOMERIC BIOMATERIAL.

Francesco De Gaetano - Supervisor: Prof. Maria Laura Costantino

In this work, an innovative biomorphic heart valve made of a new thermoplastic elastomeric biomaterial was designed, manufactured and in-vitro tested.
Nowadays only mechanical and biological heart valve prostheses are commercially available.
The former ensure long-lasting durability but anticoagulant therapy is required all life long; the latter displays better fluid dynamic performance in the short term but does not guarantee adequate durability. Fully polymeric heart valves have attracted considerable interest in the past due to their potential to combine the hemodynamic performances of biological valves with the durability of mechanical valves. This interest has waned due to the long track record of initial promises followed by failures at the preclinical stages.
In recent years, the revolutionary advance in nanotechnologies proposes novel materials very useful for the biomedical application. Block copolymers are a new polymeric class capable to tailor their mechanical properties by using different polymer fragments.
Inspired by the anisotropic architecture of collagen in the natural valve, the use of new polystyrene based block copolymers (BCPs) could be helpful to obtain a new heart valve prostheses (HVP) with the capability to mimic the oriented microstructure inside the natural aortic valve leaflet.
First, the most suitable materials and the manufacturing process capable to orient the microstructure were chosen.
Several polymers have been considered for use in the past by different research groups but only few materials could be micro and nano oriented.
Based on the literature data, three styrene BCPs were selected due to their haemocompatibility, low tendency to calcification and better inflammation response if compared to polyester and pericardium:

- poly(styrene-b-isoprene/butadiene-b-styrene), with 19% wt polystyrene fraction (SI/BS-19);
- poly(Styrene-Isoprene-Styrene), with 30% wt polystyrene fraction (SIS-30);
- poly(Styrene-Ethylene-Propylene-Styrene), with 22% wt polystyrene fraction (SEPS-22).

Mechanical tensile and small angle X-ray scattering tests were performed on these styrene BCPs to assess their properties.
A numerical model replicating the injection moulding technique was developed in order to assess the best configuration of the injection locator to well fill the cavity promoting a mainly circumferentially orientation of the micro-chains. Using simultaneous injection locator at the centre of the free edge of each leaflet, a bioinspired orientation was obtained.
From the structural point of view, a heart valve prosthesis is based on two parts: the leaflets and the stent. The leaflet is the most critical component but the stent design, especially if made of polymeric material, should not be overlooked. However, to design a polymeric HVP it is necessary to take great care also to the design of the leaflet, keeping in mind the claim for reducing regurgitation, pressure loss and enhance long-term duration of the implantation.
The shape of the leaflet could drastically influence the short-term performance (energy loss, vorticity, blood shear stress, etc.), on the other hand, the stent shape could extremely influence the long-term performances.
After selecting material, leaflet and stent geometry a mould was manufactured to produce a statistically significant number of prototypes to be tested. All the prototypes were manufactured by the research group of the Professor Geoff G. Moggridge belonging to the Department of Chemical Engineering and Biotechnology of the University of Cambridge. Both SI/BS-19 and SIS-30 were obtained starting from pellet via compression moulding, while SEPS-22 was fabricated by injection moulding.
Micro-computed tomography was used to analyse accurately the morphology of the prototypes to better understand if there are differences between the theoretical model (CAD) and the real prototypes
To assess the short-term performance of the different prototypes, hydrodynamic testing were performed following the ISO5840:2015 guidelines. An ad hoc pulse duplicator, capable of reproducing physiological flow characteristics (Fig.1), was designed and built. All the prototypes manufactured by both injection and compression moulding resulted to meet the minimum requirements in term of both EOA and regurgitation. The results were compared with one mechanical valve and one tissue valve currently on the market.
On the prototypes showing the best hydrodynamic performances, a particle image velocimetry (PIV) was performed to compare the velocity profile distribution passing through the polymeric prototypes and a bio-prosthetic heart valve, considered as a gold standard for its good fluid dynamic (Fig.2). In order to obtain significant results, all the tested prostheses had the same tissue annulus diameter.
A successful prosthesis is characterized by both good short-term fluid dynamic performance and lifelong durability. To make sure of the effective number of cycles that the prototypes could sustain, fatigue test are necessary. Quasi-real time and accelerated fatigue testing were performed on the prototypes belonging to the SEPS-22 group, showing the best short-term hydrodynamic performances. The fatigue performance was inadequate due to a manufacture imprecision which made the leaflet to be thinner than prescribed by the design. A numerical model of the polymeric valve, based on the strain energy density, was also performed. As a matter of fact, the strain energy density is a widely used criterion to predict elastomers lifetime; this result suggests a possible increase of the device durability if the polymer microstructure is optimized. The optimization of the production technique would make the proposed bioinspired styrene-based heart valve prosthesis to have the potential to overcome the issue of durability and need for anticoagulation therapy.

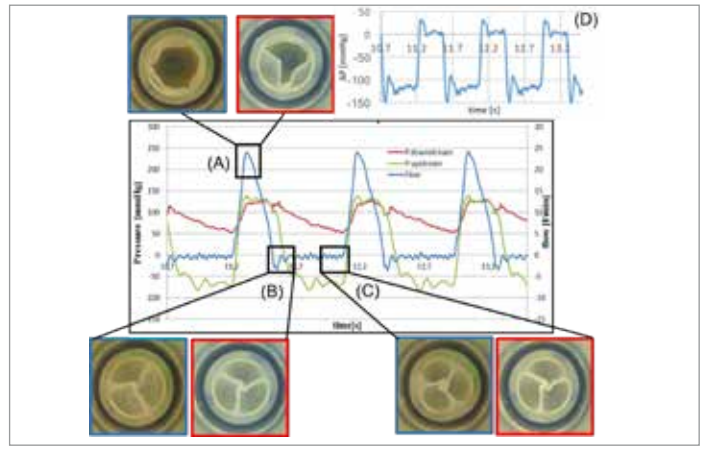

Fig. 1 - Pressure and flow courses of a generic PHV tested in this work. The pictures show the behavior of the two group of valve (blu line Group A and red line Group B) at different cycle time: (A) peak systolic flow, (B) early diastole and (C) end of diastole. Transvalvular pressure course of a generic PHV (D) is shown.

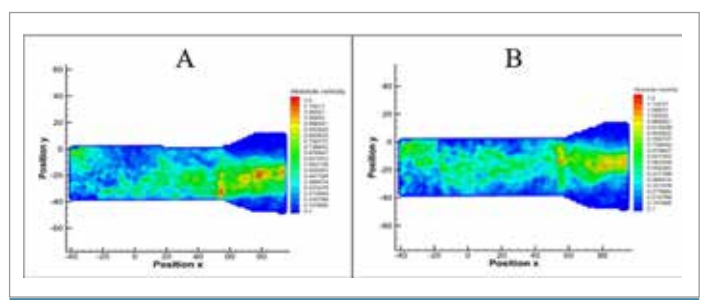

Fig.2 - Flow velocity field at 20 L/min in a prototypes belonging to the SEPS-22 Group (A) and Biological one (B).

ANTICIPATORY POSTURAL ADJUSTMENT OF GAIT INITIATION IN SUBJECTS WITH NEURO-DEGENERATIVE DISEASES: EXPERIMENTAL STUDY AND DYNAMIC MODEL SIMULATIONS

Mariangela Dipaola

Supervisors: Prof. Carlo A. Frigo, Prof. Ioannis U. Isaias

Anticipatory postural adjustments (APAs) represent a feedforward organization of a coherent set of motor commands (synergistic muscular activity) that are seen prior to an action producing a mechanical postural perturbation. Accordingly, APAs are fundamental in facilitating the set-up of the necessary condition of the forthcoming movement. This is clearly the case of gait initiation, during which the propulsive forces for the intended gait speed are generated. The study of APAs at gait initiation can therefore help understanding postural stability problems related to neurodegenerative diseases. In particular, in patients with great impairments due to disease severity, gait initiation is one of the few tasks available to investigate their locomotor behavior.

The principal aim of this study was to investigate APAs at gait initiation as a surrogate marker of *feedforward* motor control in patients with neurodegenerative disorders. We combined experimental analyses and computational simulations to identify reliable APAs measurements to support the clinical follow-up and to develop new patient-tailored rehabilitation and pharmacological strategies.

In the experimental analyses, we recorded kinematic, kinetic and electromyographic (EMG) data of gait initiation patterns in adults with neurodegenerative diseases. We used an optoelectronic system (SMART-E, BTS Bioengineering, Italy) to compute the whole body center of mass (CoM) trajectory, a dynamometric platform (KISTLER, GmbH, Winterthur, Switzerland) to measure the position of the center of pressure (CoP) and a surface EMG wireless system (FREEEMG 1000, BTS Bioengineering, Italy) for the recording of tibialis anterior (TA) and soleus (SOL) muscles.

Particularly, we enrolled 17 subjects affected by Parkinson's disease (PD) and 14 subjects with Progressive Supranuclear Palsy (PSP) (of 30 recruited) able to walk unassisted for at least three steps in medication-off condition (overnight suspension of all dopaminergic drugs). All patients had stable dopaminergic treatment for at least six months and were evaluated in the morning after overnight suspension of all dopaminergic drugs. Patients with PD were divided into two groups according to the stage of the Hoehn and Yahr scale (HY): mild group (PD_M: HY stage I or II), and severely affected group (PD_S: HY stage III or IV).

A preliminary study comparing subjects with PD_M, PD_S or PSP showed that patients greatly differ for the width of their base of support.

Therefore, we investigated in healthy control subjects (HC) the parameters of gait initiation that are influenced by anthropometrical measurements (e.g. distance between the feet) to better match patients and controls for comparisons.

Participants were instructed to stand on a force platform located in the center of the walkway, standing in self-selected upright standing position, and to start walking spontaneously after a vocal prompt.

We found that, in HC, the parameters most influenced by the variation of the distance between the feet (expressed as distance between anterior superior iliac spines, IFD%) are the lateral displacements of the CoP during all APAs phases, the distance between CoP and the CoM during APAs, the orientation of the CoP-CoM vector at the end of APAs and the first step length. The values of all these measurements increased when the IFD% also increased. The variation of the initial conditions had no effect on the position of the CoP with respect to the heels during standing and on the posterior displacement of the CoP during APAs. While both magnitude and orientation of CoP-CoM vector at the end of unloading phase were significantly dependent on ankle distance, the orientation of CoP-CoM vector at the end of imbalance phase and the CoM forward acceleration were not. These results suggests that only these two parameters are independent parameters and could be taken into account to evaluate gait initiation independently from the base of support.

Then, we re-evaluated the gait initiation abnormalities in subjects with neurodegenerative diseases (i.e. PD_M, PD_S and PSP) and this time patients were compared with different cohorts of healthy controls matched for IFD%.

Patients showed several abnormalities in APAs production and execution and lead to relevant advances in our understanding of motor control towards new patient-tailored rehabilitation and pharmacological strategies.

Particularly helpful to the interpretation of data was the evaluation of the synergic activity of distal postural muscles (i.e. tibialis anterior and soleus muscles) that resulted variably desynchronized, segmented and delayed in most of the patients.

In particular, PD_M relied mostly on the ankle plantarflexion/dorsiflexion muscles at gait initiation (i.e. ankle strategy).

Along with disease progression and possibly an increased in (axial) rigidity, PD_S developed APAs predominantly along the sagittal axis, thus suggesting a great impairment of the hip in the frontal plan and a desynchronization of ankle and hip strategies. These findings were also corroborated by an abnormal muscular activation pattern characterized by dis-synergistic or fragmented activity of pairs of postural muscles (i.e. tibialis anterior and soleus muscles).

PSP patients presented a combination of difficulties in mastering the hip and, even more, the ankle strategy as well as aberrant muscular activities, characterized mostly by a tonic activation of the TA. These measurements well correlated with the clinical picture of a devastating disease mainly characterized, at least at an early stage, by an impairment of balance with back falling. Despite we enrolled only patients able to stand and start walking unassisted; the neurodegenerative process involved in all subjects cortical and subcortical brain areas. Therefore, not only the execution of a coherent set of motor commands (i.e. APAs), but also the production (feedforward organization) of such commands was abnormal.

Finally, a musculoskeletal model for simulations of muscular activation and different type of synergies of the muscles of the lower limbs in standing position was described. This tool was used to provide some interpretation on the cause-effect relationships between the altered muscles pattern activation and the impairments observed in pathological subjects. It was found that the better strategy to push the CoM forwards was the simultaneous dorsiflexion of both ankles with comparable intensity of the torque between legs. A difference in the amplitude of the torque applied to each ankle reduced the forward push given to the CoM. On the other hand, it introduced a lateral displacement of the CoP toward the ankle on which the smaller torque acted, that caused a displacement of the CoM toward the more dorsiflexed leg. This kind of action is the strategy present in human anticipatory postural adjustments of gait initiation, when it is necessary to orient the displacement of the CoM on the stance foot. Of great relevance, the model allowed simulating conditions similar to the altered muscular synergies observed respectively in PD_M and in PD_S subjects, and presented in this work. The results we obtained with the computational simulations are in line with those observed in the experimental results and support a larger use of the musculoskeletal models in the study of biomechanics of human motion, in particular for helping the interpretation of altered pattern of movement related to neurodegenerative disease.

ADVANCED MICROFLUIDIC FLOW-BASED ASSAYS FOR SHEAR-MEDIATED PLATELET ACTIVATION IN BLOOD-CONTACTING DEVICES

Annalisa Dimasi

Supervisors: Prof. Alberto Redaelli, Prof. Gianfranco B. Fiore

Ventricular assist devices (VADs) are emerging as a valuable therapeutic treatment for advanced heart failure. Device implantation outcomes are frequently affected by thromboembolic complications, a potentially life-threatening complication. Monitoring and prevention of thrombotic events in VAD recipients is often ineffective, with limited efficacy of anti-thrombotic therapies to protect from device-related thrombosis. The development of novel approaches for evaluating platelet function *in vitro* under realistic shear stress conditions (those occurring in VADs) would represent an important advance for the development of novel and accurate flow-based assays to assess hemostatic patient conditions in realistic shear stress conditions. In this PhD study a novel approach based on microfluidics was proposed and evaluated.
In a first step, multiphase computational fluid dynamic (CFD) analyses of microfluidic channels were implemented with different aims: i) provide a numerical approach for the evaluation of the shear stress patterns along platelet trajectories in microfluidic channels; ii) demonstrate the possibility of replicating typical levels and dynamics of shear stress of mechanical circulatory support devices by modulating geometrical features of microfluidic channel and iii) provide examples of microfluidic channel designs that replicate critical patterns of shear stress of two commercial ventricular assist devices (the Heart Mate II, HMII, and the Heart Assist V, HA5 VADs). Fig. 1 provides a comparison between actual shear stress waveforms of the two VADs and the microfluidic channels designed with the developed numerical approach. The computational approach was proposed as an effective designing tool for the development of novel microfluidic systems able to replicate realistic shear stress levels and dynamics of cardiac devices.

In a second step, the validity of the CFD-based designing approach was assessed through a systematic experimental campaign in which platelet response in *ad hoc* designed microfluidic platforms was compared to a state-of-the-art system developed for dynamic controlled shear-mediated platelet activation studies. Two dynamic shear stress conditions were considered for this study (named Dyn1 and Dyn2). Platelet response in the two systems was assessed through different analyses: a chromatographic assay for measuring global pro-coagulant activity of stimulated platelet samples (PAS assay), scanning electron microscopy (SEM) acquisitions for evaluating morphological alterations of platelets, and flow cytometry analyses for evaluating exposure of a platelet activity marker (phosphatidylserine, PS). A schematics of the experimental campaign is shown in Fig. 2.

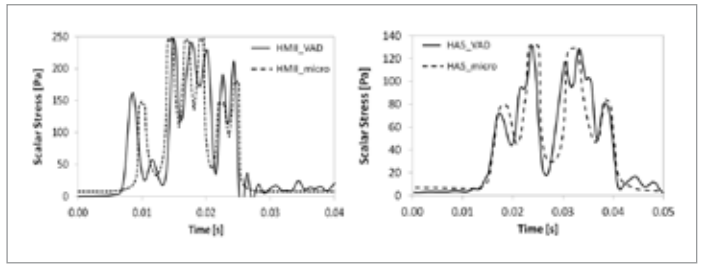

Fig. 1 - Critical shear stress waveforms of the HMII (left) and HA5 (right) VAD simulations and corresponding shear stress waveforms in the microfluidic channels.

Similar trends of platelet activation (PA) were obtained in microfluidic and HSD systems. SEM images confirmed the results of the PAS assay, showing formation of microaggregates in both HSD and microfluidic-stimulated samples at longer exposure time to shear (Fig. 3). In addition, flow cytometry provided a further insight showing analogous trends of PS exposure in both microfluidic and HSD-treated samples (Fig. 3).

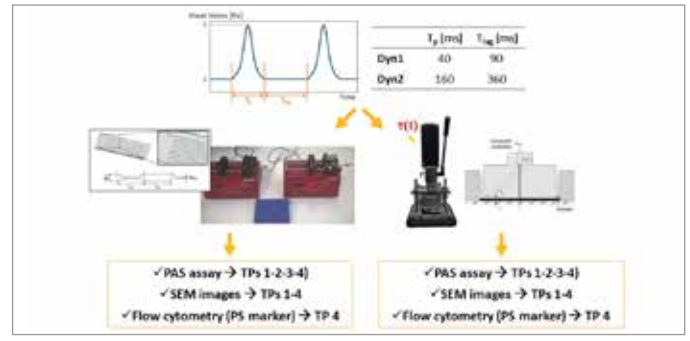

Fig. 2 - Schematics of the protocol implemented for the validity experimental campaign. Parallel tests of platelet activation in microfluidic and HSD system followed by PAS assay, SEM acquisitions and flow cytometry analyses.

Finally, the possibility of using the developed microfluidic platforms to test anti-platelet drug efficacy *in vitro* under VAD-specific shear stress conditions was evaluated. Inhibition of platelet activation treated with aspirin and ticagrelor at different concentrations was tested following exposure to VAD-like shear stress patterns in microfluidic platforms. Results suggested that the microfluidic platforms may be potentially applied to the evaluation of anti-platelet and anti-thrombotic drug regimens under realistic device-like shear flow conditions. Advancement of this technology to pre-clinical and clinical application would require integration of an *on-chip* sensing unit allowing real-time measurements of platelet activity.

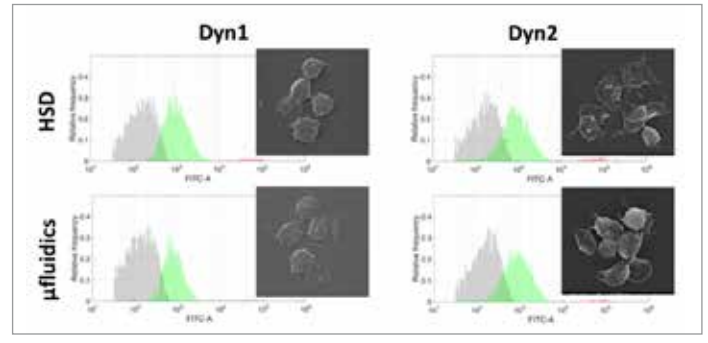

Fig. 3 - Results of PS exposure of platelets exposed to Dyn1 and Dyn2 shear stress conditions in the HSD (top) and microfluidic devices (bottom). For each condition, representative SEM images of sheared platelets are shown.

ADAPTIVE SHARED-CONTROL IN SURGICAL ROBOTICS

Nima Enayati - Supervisors: Giancarlo Ferrigno, Elena De Momi

More operating rooms are equipped with robots every year under the premise that these new tools can enhance surgical procedures. Whether robotic agents have proven to produce measurable and all-aspects-considered outcomes in the operating room or not is a matter of ongoing debate. There is, however, little doubt that the current state of surgical robotics, is far from its potential. Unlike in the industries where robots have drastically outperformed humans while acting autonomously, the role given to robotic systems in the operating room has been broadly limited to the reproduction of surgeon's commands. This is of course due to numerous technical difficulties in perception, cognition, control and actuation faced in soft tissue interaction and the complexity of surgical procedures. However, given the exponential rate of technological advancement, researchers have started preliminary works on granting a more active involvement to surgical robotic systems in recent years. The so-called shared-control approach aims at uniting the advantages of mechatronic systems with the superior cognitive abilities of humans. This work is an effort on investigating and implementing a shared-control method for surgical tele-operation where the robotic agent collaborates with a surgeon through suggestive haptic ques to enhance the operations in terms of safety, accuracy, execution time, cognitive load and surgeon fatigue. A shared-control tele-operation framework is proposed that adapts its assistive properties based on the skill-level of users estimated through a machine-learning based tele-operation skill assessment. It is shown that different operators can demonstrate disparate behaviors in using a tele-operation system and therefore, developing a one-size-fits-all assistive method may not achieve optimal results when both explicit clinical outcomes and surgeon's subjective experience are considered. It is shown here that tailoring the assistive characteristics of the system according to operator's needs can improve the human-robot interaction, which in turn can lead to better surgical outcomes. The introduced guidance method can be employed in various surgical applications where precision is of value such as instrument insertion and accurate targeting, or in operations where safety is of interest such removal of tissue in the vicinity of delicate organs. Haptic assistance can also be exploited in motor skill training to improve the learning curve of motor tasks through the reduction of cognitive load. In training of surgical tasks with complex kinematics, where it has been shown that residents can face difficulties due to poor hand-eye coordination, some form of haptic guidance can speed up the initial motor learning phase. However, there are concerns about creating assistance dependency in the trainees, which can decrease performance during the actual task. The adaptive haptic assistance introduced in this work can address such concerns by monitoring the skill level of the trainee and gradually lower the intensity and frequency of the assistance to reduce the risk of creating dependency.
This work includes experimental validation of the proposed methods. Experiments using implemented virtual environments show that the described haptic guidance enforcement method can enhance the outcome of a simplified surgical task while causing lower amount of distraction to the human operator. The skill estimation capabilities of the method is validated via experiments on a real-time teleoperation setup, where the feasibility of the adaptive guidance to is demonstrated for path following tasks.

INVESTIGATION OF ALTERNATIVE IMAGING METHODS TO IMPROVE ACCURACY IN CANCER THERAPY WITH CARBON IONS

Ferraz Dias Marta Filipa

Supervisors: Prof. Guido Baroni, Prof. Marco Riboldi

In recent years, the interest in using charged particles, such as protons and carbon ions, has shown a considerable increase with more than 140 000 patients being treated in 2015. The most common form of charged particle therapy is performed with hydrogen ions (protons). However, new facilities have been built to provide cancer treatment using carbon ions due to its higher Linear Energy Transfer (LET) and Relative Biological Effectiveness (RBE). Over the past ten years the number of patients treated with carbon ions has doubled.
The growing interest in charged particle therapy can be explained by their characteristic depth-dose curve. This shows a low and nearly flat energy deposition in the entrance point, it increases with the penetration depth until it reaches a maximum (Bragg peak) followed by a steep fall to approximately zero. In theory, when properly modeled, charged particle therapy allows the delivery of a defined dose distribution within the target volume and barely none outside of it. However, there are still many uncertainties in determining the range of charged particles inside the patient. In the clinical environment these uncertainties are accounted by increasing the target margins up to 3.5%±1mm. Such margins implicate an increase dose to nearby healthy organs, and depending on the treatment site, these can lead to severe side-effects to the patient.
Range uncertainties can come from different sources. They can be random, due to errors in patient positioning, organ motion, chemical variations in the patient tissues, and beam fluctuations. And/or they can be systematic, since treatment planning is made using X-ray Computed Tomography (CT). The range calculation of charged particles is currently performed by converting Hounsfield Units (HU) from the X-ray CT into Relative Stopping Power (RSP). Through the RSP, which measures the ratio between the tissue stopping power and water stopping power, it is possible to determine the particle's range inside the patient and place the Bragg peak within the pre-defined margins. X-rays behave very differently from charged particles when crossing matter. Therefore, the conversion between HU to RSP contains errors that in some situations can be up to 5%.
The current state-of-the-art calibration is known as the stoichiometric method, which was proposed by Schneider *et al.* To calculate the tissues RSP, the stoichiometric method requires precise knowledge of the tissue composition and ionization energy (I-value). There are different literature sources for such values, therefore there is not precise knowledge of the tissue composition and I-value, leading to uncertainties in the calibration method. Another issue with the stoichiometric method is that it only works on human-like materials. Surgical implants materials are not accounted having significantly larger errors in the range calculations. This prevents treatment through such implants since errors up to 18% can be introduced in the range calculation. A method that has been proposed to tackle these uncertainties is charged particle radiography/CT.
Charged particle radiography/CT uses charged particles with energy high enough to cross the patient. From the measured residual range/energy it is possible to obtain information about the particle's range inside the body (Figure 1).
However, charged particle radiography/CT is still limited by physical and technical constraints. To produce high quality images, correct knowledge of the particles path is necessary which can lead to long reconstruction times. Also, full coverage of the patient is necessary for charged particle CT, which requires long acquisition times and in the case of carbon imaging, it is limited by the fact that clinical accelerators are not able to accelerate carbons to energies high enough to cross all areas of the patient body. Having correct knowledge of carbon's range and interface positions in the body it is crucial for an accurate treatment.
The main aim of this dissertation was to investigate alternative methods which rely on charged particle imaging principles to reduce range uncertainties in carbon therapy.
The first method consisted in optimizing the elemental ionization values in the calculation of the RSPs to obtain a better estimation of the calibration curve. The obtained results suggest that experimental measurements of the tissue insert RSPs should be performed to obtain a list of optimized elemental I-values which will be used to compute the theoretical RSPs of other tissues.
The second method involved a phenomenological approach which predicted carbon paths trajectories better than using straight path trajectories (root mean square error was reduced by 50%).
The third method relies on multiple Bragg peak decomposition as means to obtain knowledge about the tumor edge position in a high contrast medium. The method avoided irradiation of multiple angles/positions and provided 1mm accuracy in the determination of the tumor edge.
Finally, the fourth and last method proposed the use of charged particle (protons, carbons and heliums) radiography combined with X-ray CT to obtain a patient-specific calibration curve to be used for carbon range calculations. The obtained results were extremely promising where the best results (mean RSP error under 0.7%) were obtained for helium radiography when 3 projections were used (Figure 2). The dose delivered to the patient by a single helium radiography was 8μGy, hence lower than the dose of X-ray radiography. These results suggest that helium radiography might be the method of choice for future carbon and proton treatment planning. The results derived in the different chapters of this dissertation show that carbon therapy accuracy can be increased with respect to current clinical practice.

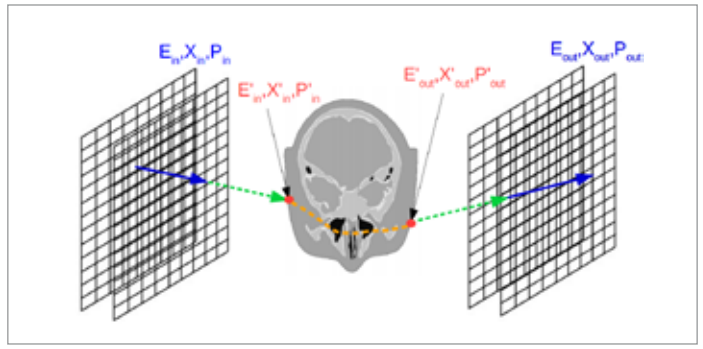

Fig. 1

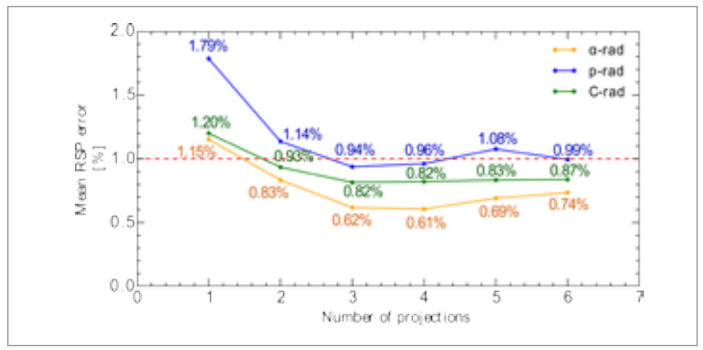

Fig. 2

CARDIORESPIRATORY PARAMETERS MONITORING BY THE USE OF CONTACTLESS TECHNOLOGY

Luca Iozzia - Supervisor: Luca T. Mainardi

In the past decades a new remote technology was presented in the scientific literature that allows to monitor physiological parameters using normal camera facing a portion of subject's skin. The present technology is called video-photoplethysmography (vPPG).

Aim

The aim of the present work is to present a robust, fully automated, remote camera-based method to investigate the potentialities of the technology in the measurement accuracy of cardiorespiratory parameters. In particular the work is composed of three main topics:
Explore the possibility to consider vPPG-based pulse rate variability (PRV) as a surrogate of ECG-based heart rate variability (HRV);
Quantification of respiratory modulation strength derived from vPPG signal and comparison of the results with a finger PPG system;
Use of vPPG system as a method to detect arrhythmic cardiac events on subjects suffering of atrial fibrillation or atrial flutter.

Background

The PPG is an electro-optic technique that measures the blood volume pulse changes through variations in transmitted or reflected light. The present technique provides cardiovascular system information as heart rate, arterial blood oxygen saturation, breathing rate and autonomic function.

The system includes the use of a dedicated light source, directly attached to the body, that illuminates the portion of the skin and a photodetector that receives the transmitted or reflected light. The evolution of PPG technology is the vPPG that, by the use of a commercial camera placed at certain distance from the skin, remotely assesses changes in the reflected light directly influenced by pulsatile changes in blood volume. Beside the absence of electrodes attached to the body, a wider spectrum of physiological analysis may be retrieved with respect to the PPG system, i.e. functional mapping perfusion state, simultaneous monitoring of different sites of the skin, and study of tidal volume. Despite the great advantages, new challenges have to be faced: the inclusion of a medium (air) in the skin-light interface introduces other undesired light sources that may corrupt the signal; the reduction of PPG signal strength due to the increased distance between the sensor and the skin; high sensitivity to motion; absence of dedicated hardware: light scattering introduced because of no uniform light source; the sensor is a normal camera with overlapping frequency color bands.

Methods

The method may be divided in three parts. The first one regards experimental setting including the choice of an industrial or commercial camera placed in front of the subject at a distance of 0.5 − 1.5 m. To simulate real scenarios a mixture of ambient and artificial light has been taken into account. Videos of the face region (used as source of PPG information) were acquired in AVI format and processed offline.
The second part consists of image processing step: an automatic face detection implemented using Viola and Jones algorithm, and face tracking through Lucas-Kanade-Tomasi (LKT) motion flow algorithm. As a final step three different rectangular regions were defined on the face: forehead, nose and cheek. For each of them a spatial average of pixel intensity was exploited at each frame to obtain raw signals. The third step was the signal post processing: a novel algorithm was presented based on zero-phase component analysis (ZCA), to enhance the pulsatile information and suppress the motion artifact eliminating the problem of correct channel selection. The proposed method was compared with two other techniques used in literature: a-priori chrominance model and independent component analysis (ICA). The present pipeline was tested in three different scenarios:
Experiment 1: A rest-to-stand experiment was conducted on 60 healthy subjects to compare the sympathetic activation through HRV obtained from ECG RR intervals and PRV obtained from vPPG pulse-to-pulse-intervals (PPI). To this extent, a preliminary analysis was carried out to assess the accuracy of pulse detection on vPPG signal comparing each pulse with the reference ECG beat.
Experiment 2: A first experiment was conducted to evaluate the breathing modulation on PPG signal, asking the subjects to breath at controlled respiratory frequencies [0.1 − 0.5] Hz with frequency step of 0.1 Hz. The breathing system affects the PPG waveform in terms of amplitude, width and pulse time occurrence. Therefore a set of methods based on pulse amplitude variability (PAV), pulse width variability (PWV) and pulse rate variability (PRV) was exploited to derive real-time breathing rate estimation. A second dataset is recorded to test the breathing rate measurement accuracy during breathing exercise: 2 minutes of spontaneous respiration followed by apnea and successive 2 minutes of recovery stage.
Experiment 3: A pool of subjects with different cardiac rhythms (atrial fibrillation, AF, atrial flutter, AFL, and normal sinus rhythm, SR) was recruited to test the feasibility of vPPG system as method to detect cardiac arrhythmic events. A set of features was extracted from PPI series: SDNN, RMSSD, sample entropy and the ratio SD1SD2 that expresses the spatial distribution of PPI in Lorenz plot. Beside them, two novel parameters were introduced: the power harmonic strength (PHS), index of percentage of energy located around the dominant frequency of vPPG spectrum respected to the total spectrum energy, shape similarity (SS) that measures the PPG wave similarity along time running windows. A feed forward neural network (FFNN) classifier was trained using the most relevant features (selected by the ANOVA statistical method) in order to discriminate the three different categories of rhythm.

Results

The results are presented according to each experiment:
Experiment 1: The postural change from rest to stand provokes a slight decrease both of signal-to-noise ratio of vPPG signal (average decrease of 6.75 dB) and of the total accuracy of pulse detection (0.984 ± 0.021 % at rest vs 0.979 ± 0.023 % in a standing position with no statistical significance). The main rea- son is the increase of motion noise introduced by the subject to maintain the balance.
Pearson correlation was used to compare temporal and frequency domain parameters between PRV and HRV. In rest condition, both temporal and frequency features maintained a correlation greater than the 0.9 threshold. In the standing condition, only SDNN had a high correlation, while the other parameters (RMSSD, LF, HF) had a drop in concordance. The decrease of correlation between the two systems in the standing condition explains the moderate change in the LF/HF ratio found in PRV (0.49 ± 0.34 in rest vs 0.70 ± 0.51 in standing) while in HRV the sympathetic activation is more evident (0.51 ± 0.38 in rest vs 1.17 ± 0.79 in stand).
Experiment 2: The results are expressed as median and interquartile ranges of relative error calculated between the estimated breathing frequency by vPPG and the gold standard respiratory belt. In the first database a good perfomance in the estimation of breathing rate was found in the respiratory frequency range [0.2 − 0.4] Hz (0.89/3.03 % expressed as median/IQR). The relative error increased dramatically at the respiratory frequencies boundaries (f_R = 0.1 Hz with −8.80/35.69 % and at f_R = 0.5 Hz with 38.65/36.55 %). In the second database the relative error remained acceptable both during spontaneous breathing (0.43/2.11 %) and during the recovery stage (0.46/4.81 %).
Experiment 3: Among all the features used, only PHS and SS displayed a statistically significant difference between the three classes SR, AF and OA (class containing AFL rhythm). The general performance of the FFNN is 85.9 %, that represents the percentage of the total correct predictions. The main drop of performance regarded the misclassification between the two classes AF and OA. Among 55 patients suffering of AF or AFL, 12% of subjects are labeled incorrectly. Reducing the complexity of classification to only two classes, SR and arrhythmias, the classifier improves its performance to 95%.

Conclusion

A fully automated method has been presented to detect remotely physiological parameters. Under stationary conditions (reduced subject motion) the system shows its reliability to measure pulse rate, PRV, breathing rate and cardiac patterns irregularities. Significant improvements are mandatory to create a robust system under more real conditions that imply largely subject movements and scenarios in which the face is poorly recognizable. Once the technology demonstrates its reliability in a wider spectrum of applications, new markets opportunities will be available, from elderly telemonitoring to infant monitoring.

PANCREATIC ISLET MICROENCAPSULATION: DEVELOPMENT OF A COMPREHENSIVE PLATFORM FOR EVALUATION AND OPTIMIZATION OF CONFORMAL COATING WITH HYDROGELS FOR CLINICAL APPLICATIONS

Vita Manzoli - Supervisor: Prof. Alberto C. L. Redaelli

Pancreatic islet transplantation aims at replacing β-cells, targets of autoimmune attacks in type 1 diabetes mellitus. To prevent graft rejection, patients require chronic immunosuppression, which is not completely effective and can cause adverse events. Therefore, islet transplantation is currently performed only in the most severe cases. Immunoisolation through islet encapsulation may allow transplantation without immunosuppression, but clinical success has yet to be achieved.

The aim of this PhD dissertation is to present a new encapsulation device for conformal coating of islets with PEG-based polymers through a micro fluidics device. The work presented focuses on overcoming some limitations of traditional islet microencapsulation by: i) reducing the volume to be transplanted by decreasing capsule thickness; ii) enhancing capsule stability by using PEG-based polymers, iii) optimizing the transplantation site in order to favor encapsulated islets engraftment, iv) optimizing coating composition by enhancing PEG-hydrogels with ECM components, v) developing methods to allow high-throughput screening of candidate materials for conformal coating using computational models and lab-on-chip technology, vi) proving proficiency of conformal coating to cell therapy approaches in contexts different from diabetes.

Thin conformal coatings (Figure 1) were obtained using a fluid dynamic approach: the water jet forming between two immiscible fluids (oil and water) flowing coaxially gets broken through the use of a flow-focusing geometry. The obtained droplets consist of islets covered in a thin polymer layer. The fluid dynamic process was first studied in silico through a computational model and then evaluated in vitro by comparing prototype devices.

Islets were encapsulated using polyethylene glycol-divinyl sulfone supplemented with alginate and cross-linked with dithiothreitol (PEG ALG). The encapsulation did not compromise viability and function both *in vitro* and *in vivo* in a syngeneic murine transplantation model at the renal sub capsular site (KD).

A study about the effects of capsule composition and transplant site on microencapsulated islet graft outcomes in mice was carried out. The epididymal fat pad (EFP) proved to be a superior site in terms of long-term diabetes reversal. Addition of PEG to alginate to form hybrid microcapsules improved diabetes reversal time in the peritoneal cavity site, most likely by conferring higher mechanical protection to the capsules. This was not the case in the EFP site, where the lower biocompatibility of the hybrid capsule might have a played a role in longer reversal time and poorer glycemic control. PEG ALG coatings failed to achieve long-term normoglycemia in an allotransplantation context. This might have been related to their microporous structure. In order to improve transplantation outcomes, PEG divinyl sulfone functional groups were replaced with the less reactive maleimide groups, and the KD site with the EFP site. ALG was replaced by Matrigel (MG), in order to recapitulate the islet-like extracellular matrix component. Immunoisolation and long-term diabetes reversal were achieved transplanting PEG MG conformally coated islets in a fully MHC-mismatched allotransplantation model in mouse in absence of immunosuppression.

Steps toward clinical translation were taken by implementing further refinements to the coating composition and transplant site and by developing a computational model of the transplant site. Engineered fibrin gels used to anchor the islets to the EFP were replaced by a biologic scaffold (autologous plasma of the transplant recipient mixed with recombinant thrombin). The scaffolds were found to perform comparably *in vivo* in a syngeneic transplant model. Also, Matrigel (not clinically translatable) was replaced with a synthetic polymeric peptide, PepGel. Concerns about dithiothreitol toxicity led to replace it with a 2KDa PEG-dithiol. The materials were first evaluated from a biologic perspective (coating completeness, viability and function in vitro and in vivo in a syngeneic mouse transplantation model) and then adopted as reference material in a computational model of the conformally coated islet in the EFP transplant site. The model allows to study transport phenomena of glucose from the blood vessel to the center of the islet and of insulin in the opposite direction. The total delay between blood glucose increase and insulin release in the bloodstream in the transplantation site could be predicted using the model. The parametric nature of the model also made it possible to compare different capsule compositions. Finally, an application of conformal coating in a context different than diabetes was shown. Human renal epithelial cells were used as a cell model to prove efficacy of conformal coating in potentially endless cell transplantation therapies for regenerative medicine.

Overall, the presented work shows great promise for successful translation of the conformal coating technology in clinical applications.

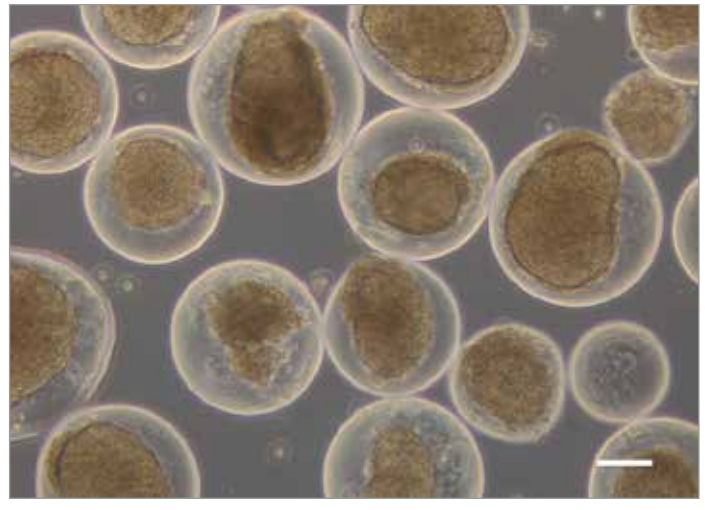

Fig. 1 - Conformally coated pancreatic islets (Scalebar: 100µm)

ASSESSMENT OF VENTRICULAR REPOLARIZATION INSTABILITY AND CARDIAC RISK STRATIFICATION IN DIFFERENT PATHOLOGICAL AND ABNORMAL CONDITIONS

Alba Pilar Martín Yebra

Supervisors: Enrico Gianluca Caiani, Juan Pablo Martínez Cortés

Cardiovascular diseases represents the leading cause of mortality worldwide. These pathological conditions are mainly characterized by a structurally abnormal heart, that is, a vulnerable substrate, prone to the abnormal generation and/or propagation of the electrical impulse, determining the onset of ventricular arrhythmias. In this context, the assessment of ventricular repolarization from the electrocardiogram (ECG) signal has been shown to provide with valuable information for risk stratification and several electrocardiographic indices have been proposed in the literature.

The main objective of this thesis is to propose methodological advances for the assessment of ventricular repolarization instability in pathological and abnormal conditions. These contributions are aimed at improving the prediction of ventricular arrhythmias and, consequently, better identifying sudden cardiac death (SCD) risk.
In particular, we have addressed this objective by developing robust methodologies for the assessment of T-wave alternans (TWA) and ventricular repolarization instability, in invasive and non-invasive cardiac signals, that have been evaluated in both experimental and clinical conditions.

In the first part of the thesis, TWA was simultaneously characterized (prevalence, magnitude, time-course, and alternans waveform) in bodysurface ECG and intracardiac electrograms (EGMs) signals during coronary artery occlusion. Signals from both body surface ECG and intracardiac EGMs recorded from 4 different anatomical heart locations (coronary sinus, epicardial space and left and right ventricles) were analyzed following a multilead strategy. Leads were linearly combined using the periodic component analysis (πCA), which maximizes the 2-beat periodicity (TWA periodicity) content present on the available leads. Then the Laplacian Likelihood Ratio method (LLRM) was applied for TWA detection and estimation. A sensitivity study for TWA detection from the 5 different locations of leads was performed, revealing that it is the combination of the ECG leads that better performs. In addition, this multilead approach allowed us to find the optimal combination of intracardiac leads usable for in-vivo monitorization of TWA directly from an implantable device, with a sensitivity comparable to the ECG analysis. These results encourage further research to determine the feasibility of predicting imminent VT/VF episodes by TWA analysis implemented in implantable cardioverter defibrillator's (ICD) technology.
Then, we have studied the potential changes induced by a prolonged exposure to simulated microgravity on ventricular repolarization in structurally normal hearts. It is well known that this environmental condition affects the control of autonomic and cardiovascular systems, with a potential increase on cardiac electrical instability. The effects of short- (5 days), mid- (21 days) and long- (60 days) exposure to simulated microgravity on TWA using the head-down bed-rest (HDBR) model were assessed. TWA was evaluated before (PRE), during and after (POST) the immobilization period, by the long-term averaging technique in ambulatory ECG Holter recordings. Additionally, we proposed an adapted short-term averaging approach for shorter, non-stationary ECG signals obtained during two stress manoeuvres (head-up tilt-table and bicycle exercise tests). Both approaches are based on the multilead analysis used in the previous study. The absence of significant changes between PRE and POST-HDBR on TWA indices suggests that a long-term exposure to simulated microgravity is not enough to induce alterations in healthy myocardial substrate up to the point of reflecting electrical instability in terms of TWA on the ECG.
Finally, methodological advances were proposed for the assessment of ventricular repolarization instability from the ECG signal in the presence of sporadic (ventricular premature contractions, VPCs) and sustained (atrial fibrillation, AF) rhythm disturbance.
On the one hand, a methodological improvement for the estimation of TWA amplitude in ambulatory ECG recordings was proposed, which deals with the possible phase reversal on the alternans sequence induced by the presence of VPCs. The performance of the algorithm was first evaluated using synthetic signals. Then, the effect of the proposed method in the prognostic value of TWA amplitude was assessed in real ambulatory ECG recordings from patients with chronic heart failure (CHF). Finally, circadian TWA changes were evaluated as well as the prognostic value of TWA at different times of the day. A clinical study demonstrated the enhancement in the predictive value of the index of average alternans (IAA) for SCD stratification. In addition, results suggested that alternans activity is modulated by the circadian pattern, preserving its prognostic information when computed just during the morning, which is also the day interval with the highest reported SCD incidence. Thus, suggesting that time of the day should be considered for SCD risk prediction.
On the other hand, the high irregularity of the ventricular response in AF limits the use of the most common ECG-derived markers of repolarization heterogeneity, including TWA, under this clinical condition. A new method for assessing ventricular repolarization changes based on a selective averaging technique was developed and new non-invasive indices of repolarization variation were proposed. The positive impact in the prognostic value of the computed indices was demonstrated in a clinical study, by analyzing ECG Holter recordings from CHF patients with AF. To the best of our knowledge, this is the first study that attempts a non-invasive SCD stratification of patients under AF rhythm by assessing ventricular repolarization instability from the ECG signal.
To conclude, the research presented in this thesis sheds some light in the identification of pro-arrhythmic factors, which plays an important role in adopting efficient therapeutic strategies. In particular, the optimal configuration for real-time monitoring of repolarization alternans from intracardiac EGMs, together with the prognostic value of the proposed non-invasive indices of alternans activity and ventricular instability variations in case of AF rhythms demonstrated in two clinical studies, would increase the effectiveness of (ICD) therapy. Finally, the analysis of ECG signals recorded during HDBR experiments in structurally healthy hearts, also provides interesting information on cardiovascular alterations produced in immobilized or bedridden patients.

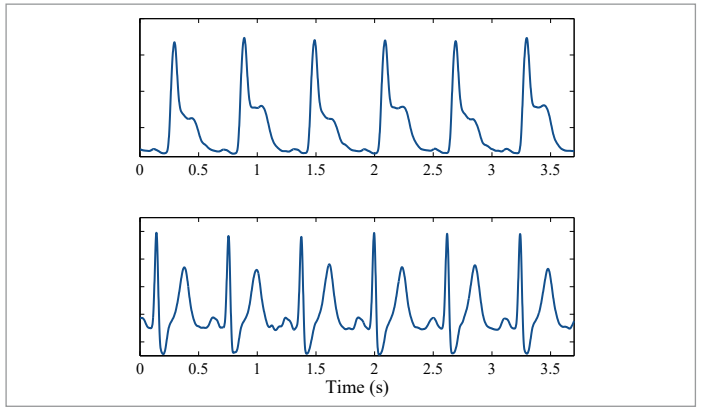

Fig.1 - Two examples of ECG signals with TWA

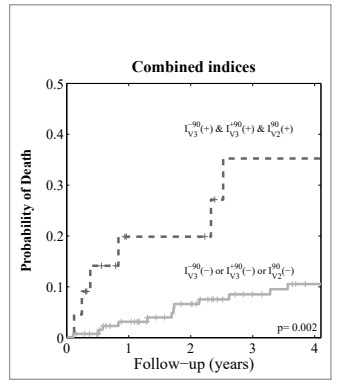

Fig. 2 -Kaplan-Meier curve for SCD incidence associated to proposed indices.

STUDY OF COMPUTER VISION ALGORITHMS TO ENHANCE THE SURGEON'S CAPABILITIES IN ROBOTIC MINIMALLY INVASIVE SURGERY

Veronica Penza

Supervisors: Prof. Elena De Momi, Prof. Leonardo Mattos

Background

Minimally Invasive Surgery (MIS) has revolutionised the traditional open-surgery technique by reducing the invasiveness of the access to the surgical site inserting surgical instruments and endoscope through few small incisions, reducing patient's trauma, risk of infections, and thus, improving the surgical outcome. Despite the benefits, the uptake of MIS surgery has been slow due to some limitations, including limited surgeons' manoeuvrability, reduced haptic and depth perception, limited freedom of movement due to the single endoscope port access and limited field of view of the surgical scene. The introduction of Robotics in Minimally Invasive Surgery (RMIS) overcomes many of the obstacles introduced by traditional laparoscopic techniques, by improving the surgeon's manoeuvrability, the precision, and restoring hand-eye coordination and depth perception during the surgical procedure. Nevertheless, even if surgeons can benefit from such advanced technologies, the core of the surgery still relies on their degree of expertise and experience, and on their ability, for example, to fuse pre-operative information intra-operatively, making the outcome of the surgery depending on inter-surgeon skills. In abdominal surgery, for example, intra-operative bleeding is one of the major complications that affects the outcome of minimally invasive surgical procedures. One of the causes is attributed to accidental damages to arteries or veins, and one of the possible risk factors falls on the surgeon's skills.

Aims

The overall goal of this thesis is to develop novel computer vision algorithms for the implementation of assistive technologies focused at enhancing the surgeon's intra-operative capabilities, to allow safer clinical procedures and improved outcomes. As a clinical application, we decided to focus the attention on abdominal surgery, where augmented reality can have a large impact and the challenges for its implementation are many and still open.

Methods

The PhD research has been focused on the following topics: (I) Development of a dense 3D reconstruction algorithm to intra-operatively measure soft tissue deformations, robust to illumination-variations, specular highlights and tissue characteristics. The work is focused on the implementation of a dense and accurate 3D reconstruction algorithm enriched with a novel refinement disparity map strategy, based on Simple Linear Iterative Clustering (SLIC) super pixel technique. In order to evaluate the algorithm, a phantom of abdomen (including liver, kidneys and spleen) was developed and a new and rich stereo endoscopic image dataset (EndoAbS dataset) was created. This dataset is openly available for the benefit of the computer vision community. Results show an accuracy of the algorithm below 2mm, with a percentage of reconstructed points over 70%, complying with the clinical requirements; (II) Development of a long-term safety area tracker (LT-SAT) to preserve soft tissue areas from injury during surgery. The proposed framework combines an optical flow algorithm with a tracking-by-detection approach in order to be robust against failures caused by: (i) partial occlusion, (ii) total occlusion, (iii) Safety Area (SA) out of the field of view, (iv) deformation, (v) illumination changes, (vi) abrupt camera motion, (vii) blur and (viii) smoke. A Bayesian inference-based approach is used to detect the failure of the tracker based on online context information. A Model Update Strategy (MUpS) is also proposed to improve the SA re-detection after failures, taking into account the changes of appearance of the SA model due to contact with instruments or image noise. Results obtained from test on ex-vivo and in-vivo video datasets show high precision (0.85) and recall performance (0.6), even in long videos (≃ 4min); (III) Development of an Enhanced Vision System to Improve Safety in Robotic Surgery (EnViSoRS) to protect vessels from injury during the execution of a robotic surgical procedures. The core of the framework consists in the integration of the previously developed dense 3D reconstruction algorithm (I) and long-term tracking (II), in combination with Augmented Reality (AR) features for warning the surgeon about distance between the instruments and a vessel enclosed by the SA. Results demonstrate that the proposed system has potential to offer useful assistance during real surgeries with commercial surgical robot (the dVRK system).

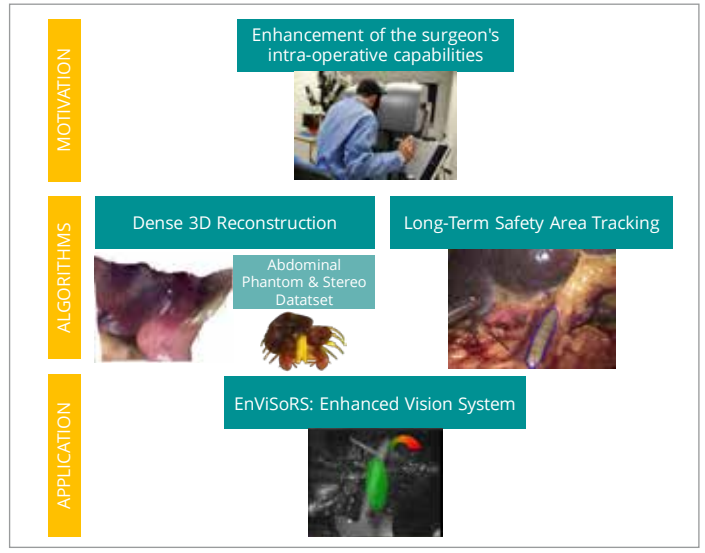

Fig. 1 - Workflow of the thesis. The motivation is the enhancement of surgeon' capabilities during robotic minimally invasive surgery. Computer vision algorithms were developed to reconstruct the surgical site and track soft tissues. An application of such methods combined with augmented reality features was developed to protect delicate areas from injuries during robotic surgery.

Conclusion

The overall results presented by this thesis demonstrates the feasibility of using computer vision algorithms to enhance the surgeon's capabilities during the execution a surgical procedure. The methodological progresses made in this work stress the potential of such algorithms in exploiting and extracting useful information implicitly contained in the images, overcoming challenging issues typical of an endoscopic surgical scenario and being inline within the requirements in terms of accuracy. In the future perspective, the proposed work represents only a portion of a wider framework to support the surgeon in the different phases of surgery.

EVIDENCE-BASED METHODOLOGIES TO ASSESS THE EFFECTIVENESS OF NOVEL TECHNOLOGIES FOR NEUROREHABILITATION

Elisabetta Peri - Supervisors: Prof. Alessandra Pedrocchi, Prof. Simona Ferrante

Neurological diseases affect an increasing number of individuals, both in adult and children populations. With the growing field of technology, plenty of different tools are available to promote long-term neuromotor recovery, but their effects and potential benefits are still poorly investigated. From a patient's perspective, what is primarily important is receiving the best treatment available for the recovery of functional abilities and independence in daily life activities.

In this framework, the research activity carried out during my Doctoral program was primarily intended to identify which rehabilitation option is more effective among the available ones, and to propose new rehabilitative approaches specifically aimed at maximising long-term recovery, triggered by neuroplastic changes. Furthermore, technology-based tools have been proposed to assess the induced changes. They were exploited to evaluate rehabilitative protocols and to investigate neural correlates of functional recovery, enhancing the knowledge of the recovery mechanisms underlying rehabilitation.

In the present thesis, different technologically-advanced tools commonly used clinical practice has been investigated as tools to promote rehabilitation and/or to evaluate the rehabilitative effects on children affected by cerebral palsy (CP) and stroke population. First, upper and lower limb robot-assisted rehabilitation paradigms have been studied on children affected by CP as few, weak and inconsistent evidence on robotics effects was shown in literature in the paediatrics population, although it is commonly used in clinical practice. A non-operator dependent, quantitative performance parameter computed from data extracted by an upper limb robotic device (Armeo Spring, Hocoma) has been proposed. This tool was able to quantitative evaluate motor ability as well as to follow the patients performance during robotic treatment. Two clinical spillovers of this study were: i) the use of the performance parameter to plan the robotic intervention in clinical practice; ii) the possibility to update session by session the training of each patient on the bases of the performance achieved. The proposed parameter was used, together with functional scales, in the first clinical study published on CP children trained with Armeo Spring. Ten children affected by cerebral palsy (age 8-15) underwent 20 sessions of training of proximal arm with Armeo®Spring. In addiction, they received 20 sessions of technology-assisted training focused on hand/finger fluency and dexterity in a pre-post treatment experimental design. Results showed significant improvements in upper limb coordination and fluency, with a good transferability to everyday life also in areas not specifically trained.

Robot Assisted Gait Training (RAGT with Lokomat, Hocoma) has been studied in order to provide information about the best treatment scheme. The effect of type of intervention (task-oriented physiotherapy vs RAGT), duration (4 vs 10 weeks) and frequency (4 sessions/week vs 10 sessions/week) was investigated in forty-four children affected by CP who underwent equal dose of intervention. Results assessed that single-treatment approaches seem to be more effective than mixed approaches, independently from the duration of treatment. RAGT seems to have similar effects with respect to the traditional physiotherapy, at least over 10 weeks. An investigation on the role of RAGT on CP patients' metabolic activity during every-day life has also been proposed in a case-series study, suggesting promising results in terms of increased level of physical activity and enhanced locomotion efficiency, in accordance to the healthy group. The peculiarity of this study was that the data were acquired in an ecological setting, minimizing possible bias due to environmental conditioning of hospital and clinicians.

Part of my research activity was also focused on stroke population. Recent studies advocated the use of technologically advanced intervention to facilitate neuroplasticity and enhance functional improvements in stroke adult patients. A research activity of the present thesis was aimed at assessing if an innovative intervention involving a biofeedback cycling training augmented by Functional Electrical Stimulation (FES) together with biofeedback balance training, is superior to usual care in improving motor recovery soon after stroke. The hypothesis is that early interventions based on motor relearning principles, but not directly involving locomotion, may improve walking abilities. I proposed and evaluated the effects of this innovative with respect to usual care with a single-blind randomized control trial carried out on post-acute stroke subjects, suggesting the superiority of the innovative training paradigm in terms of performance during dynamic balance. Differently, the two training paradigms seem to be equivalent in terms of clinical scales, gait and cycling performance.

Finally, the reliability of transcranial magnetic stimulation as a method to assess possible changes in terms of corticospinal excitability has been investigated. Stimulus-Response (SR) curves have been acquired with a recently proposed rapid protocol on tibialis anterior muscle of healthy older adults. A test-retest protocol was carried out in order to evaluate the intra and inter session reliability of TMS measures derived from SR curves collected on twenty-four neurologically-intact older adults (age 55-75 years). The results obtained suggest that measures derived from SR curves acquired in less than four minutes are affected by similar measurement errors to those found with long-lasting protocols. Therefore, the rapid acquisition method is at least as reliable as the traditional acquisition method.

To conclude, the results reported in the present thesis step a little forward the knowledge about the efficacy of technologically-advanced treatments in the field of neurorehabilitation, toward evidence-based medicine. The assessment tools proposed offer clinicians flexible, quantitative and operator-independent methodologies to investigate possible benefits obtained by neurorehabilitation. At the same time, clinical trials give an insight on the use of novel technologies in clinical practice.

Figure 1 provides an overall schematic representation of the main outcome achieved by the present thesis and its most relevant spillovers in clinical practice. Possible future research directions are also suggested.

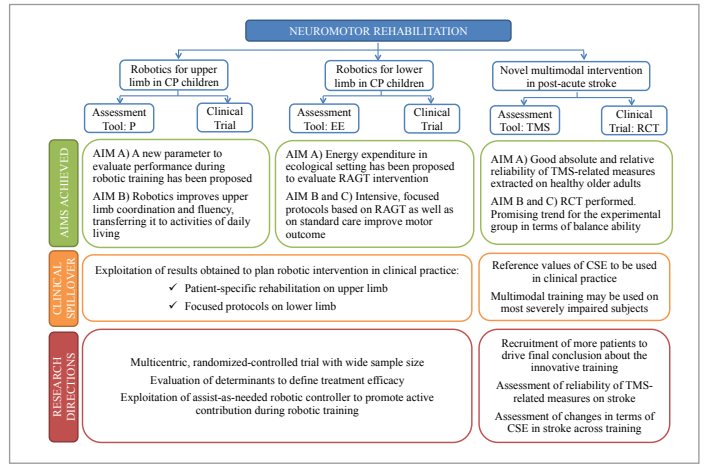

Fig. 1 - Schematic representation of the main outcome, clinical spillovers and future research are shown. RAGT: robot-assisted gait training; EE: energy expenditure; CSE: Corticospinal Excitability; TMS: transcranial magnetic stimulation.

FLOW ENCODING MAGNETIC RESONANCE IMAGING: DEVELOPMENT OF ADVANCED COMPUTATIONAL METHODS FOR THE ASSESSMENT OF IN VIVO FLUID DYNAMICS

Filippo Piatti - Supervisor: Redaelli Alberto, Votta Emiliano

Recent developments in flow encoding magnetic resonance imaging consisted in the definition of novel 3D time-resolved phase-contrast cardiac magnetic resonance pulse sequences with three-directional velocity-encoding. These imaging technique, namely 4D Flow, allowed for capturing complex 3D time-resolved velocity patterns for retrospective investigations of any location within a volume of interest. Several studies proved the potential of 4D Flow in comprehensively describing the in vivo fluid dynamics on a patient-specific basis, so to overcome the inherent limitations of in silico modeling approaches and in vitro testing set-ups. Nevertheless, from a technical standpoint, the reliability of 4D Flow sequences is still under investigation, considering their current limitations in terms of spatial resolution (1.5-3 mm); temporal discretization of the cardiac cycle (30-50 ms); and lack of standardized post-processing approaches. The aforementioned limitations hamper the reliability of estimated hemodynamic markers and consequently the application of 4D Flow sequences in the clinical scenario, especially considering those clinical scenarios where the biomechanical implications of altered fluid dynamics on the onset and progression of cardiovascular diseases are yet unknown. In this specific context, three major challenges were tackled: i) to improve and assess the reliability of 4D Flow derived markers, e.g., wall shear stresses, through the definition of realistic in silico set of 4D Flow datasets; ii) to test the relevance of 4D Flow in the analysis, risk stratification and monitoring of diseases affecting the thoracic aorta; iii) to investigate the feasibility of 4D Flow in quantifying the complex 3D fluid dynamics of blood processing devices, in sight of being a valid tool for devices optimization. Considering the first topic, two new methods were developed for the quantification of wall shear stress (WSS) acting on the aorta. The extensive benchmarking highlighted the need for data spatial resolution at least comparable to current clinical guidelines, the low sensitivity of the methods to data noise, and their capability, when used jointly, to compute more realistic peak WSS values as compared to state-of-the-art methods. In light of the improved estimation of shear forces acting on the endothelium of the aortic wall, a specific clinical scenario was taking in consideration to assess the role of WSSs in the progression of aortopathy related to the presence of bicuspid aortic valve and to the associated altered fluid dynamics. To test this hypothesis, 4D Flow was used to analyze the in vivo fluid dynamics in the thoracic aorta of normo-functional BAV patients with no aortic dilation. Despite minor bulk flow disturbances at peak systole, evident alterations of WSS distribution and peak values, and WSS-related indexes were found. Importantly, no clinically relevant anatomical remodeling was observed in a three-year follow-up, suggesting that WSS alterations may precede the onset of aortopathy and may contribute to its triggering (Figure 1).

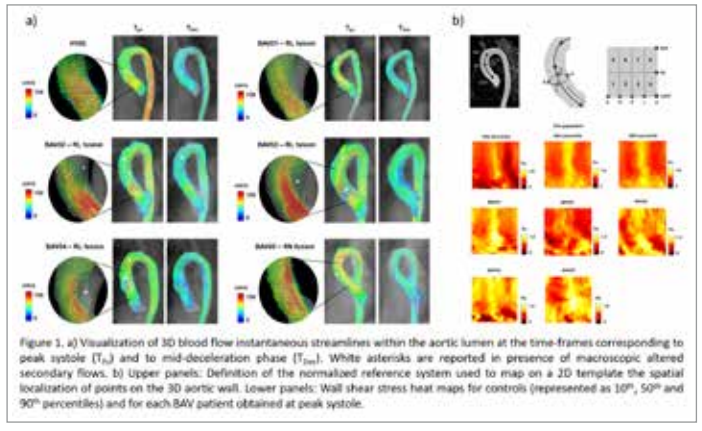

Figure 1. a) Visualization of 3D blood flow instantaneous streamlines within the aortic lumen at the time-frames corresponding to peak systole (T_{PS}) and to mid-deceleration phase (T_{DEC}). White asterisks are reported in presence of macroscopic altered secondary flows. b) Upper panels: Definition of the normalized reference system used to map on a 2D template the spatial localization of points on the 3D aortic wall. Lower panels: Wall shear stress heat maps for controls (repeesented as 10th, 50th and 90th percentiles) and for each BAV patient obtained at peak systole.

Moreover, 4D Flow was exploited to assess in vivo differences in aortic flow dynamics after valve sparing root replacement, with and without reconstruction of the sinuses of Valsalva and in matched controls. Physiologic-like sinus vortices were clearly visible in the aortic root when using the prosthesis with Valsalva neo-sinuses while the straight tubular graft revealed localized intrados malrotations. In the ascending aorta, recreation of the sinuses of Valsalva resulted in significantly lower values of velocity and WSS. Also, lower WSSs in the distal thoracic aorta is a novel finding with potential implications on distal aortic remodeling (Figure 2).

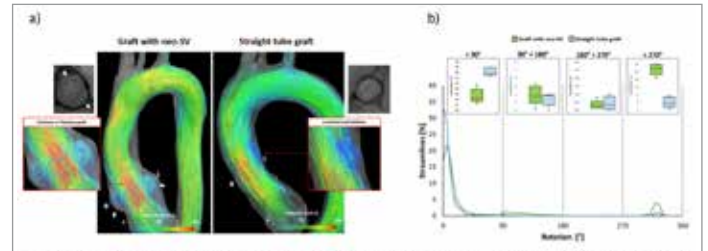

Figure 2. a) 3D instantaneous streamlines describing the bulk flow field at peak systole for two patients implanted with a graft with neo-SV and a straight tube graft, with a focus on the vortical structures within the prostheses. The cineMR planes passing through the aortic root are also reported. b) Frequency distribution of the in-plane streamlines according to the respective amount of rotation, i.e., the angle spanned by each streamline. Box-plots represent the distribution of data as observed when clustering the streamlines in four sub-groups.

As regards the third topic, 4D flow sequences with sub-millimetric spatial resolution and region-dependent velocity encodings were tested on a real device that integrates an oxygenator and a heat exchanger. The effects of fine geometrical features of the device on the local fluid-dynamics were highlighted, these phenomena being unlikely measurable by current in vitro approaches. Also, the effects of non-idealities on the flow field distribution were captured, thanks to the absence of the simplifying assumptions that typically characterize in silico approaches. These evidences proved the ability of 4D Flow mapping in extracting sound information on the fluid-dynamic performances of blood processing devices (Figure 3).

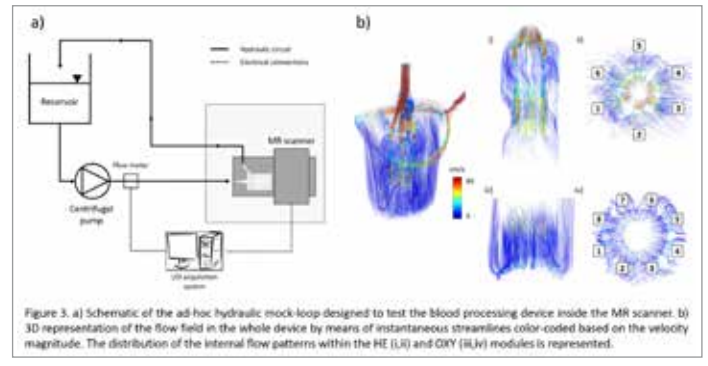

Figure 3. a) Schematic of the ad-hoc hydraulic mock-loop designed to test the blood processing device inside the MR scanner. b) 3D representation of the flow field in the whole device by means of instantaneous streamlines color-coded based on the velocity magnitude. The distribution of the internal flow patterns within the HE (i,ii) and OXY (iii,iv) modules is represented.

DEVELOPMENT AND VALIDATION OF AN EYE TRACKING SYSTEM FOR PROTON RADIOTHERAPY TREATMENT OF OCULAR MELANOMAS

Riccardo Via - Supervisor: Prof. Guido Baroni

Ophthalmic tumors are rare, but potentially fatal lesions, with enucleation being the most common choice of therapy before radiotherapy, a more globe-conserving approach, gained prominence in the 1970's. The progress of external beam radiation therapy (EBRT) and brachytherapy shifted therapeutic choices on the evidence of no unfavorable effect on survival rate. Ocular brachytherapy is differentiated by the choice of radiation source (106Ru/106Rh or 125I plaques) featuring different dose distributions in the ocular globe, with the radiation oncologist's choice being determined by the tumor geometry and site. A high mean local control of 90.1% has been reported even though typically associated with radiation-induced complications, including cataract, retinopathies and optic neuropathies, potentially leading to severe functional loss despite the successful outcome of the treatment. Intraocular lesions ineligible for brachytherapy have been treated with radiation therapy using photons and protons. Although, Linac-based stereotactic radiotherapy (SRT) has been used to treat intraocular lesions with a 3-year local control rate higher than 96% and an overall eye retention of 86% at 5 years, the improved dose conformity of proton therapy has made this modality the most favorable therapeutic option for juxta papillary tumors and lesions with thicknesses larger than 7 mm. Treatment planning comparisons between SRT and ocular proton therapy (OPT) suggest increased homogeneity in the target and complete sparing of contralateral organs at risk with proton planning while showing no significant changes in target dose conformation. As of 2014, proton therapy has been successfully delivered to over 28,000 patients affected by various kinds of intraocular lesions. Five-year tumor local control and eye retention, reported by different institutions, ranged between 91%-96% and 75%-91%, respectively. These positive outcomes are counterbalanced by proton radiation effects on the patient's visual acuity which, according to literature, deteriorate in 33-47% of cases if the lesion is located near the macula or the optic disk.

Ocular proton therapy however requires an invasive surgical procedure in which radio-opaque tantalum markers are sutured onto the sclera of the diseased eye. The surgeon performs geometrical measurements of the lesion dimension and position, and annotates the distance with respect to the limbus and the tantalum markers. Clinical ultrasound data and fundus photographs of the diseased eye complete the diagnostic dataset, which is then fed to a dedicated treatment planning system, EYEPLAN. From this, EYEPLAN creates a geometrical model of the eye and tumor, from which the optimal eye gaze angle for the given tumor location can be determined and the corresponding dose distribution to the target and critical ocular structures calculated. To mitigate the effect of uncertainties a safety margin, typically 2.5 mm, is adopted. During the treatment, the selected gaze angle is assured by the patient concentrating on a fixed light diode, correctly positioned in front of the treated eye. As such, centers offering OPT are typically equipped with a dedicated fixed proton beam line and treatment specific in-room imaging devices, consisting of orthogonal X-ray imaging systems for treatment geometry verification. Setup corrections are estimated by comparing the position of the implanted tantalum markers in orthogonal radiographies to their reference location estimated by the TPS, with the patient position being corrected using a robotic treatment chair. Typically, multiple iterations of this procedure are required to achieve an accurate target-beam alignment. Subsequently, and during treatment delivery, the eye is monitored simply by visual control of a CCTV camera pointed at the treated eye. To guarantee the reproducibility of the gaze direction during treatment, the radiation technologist must outline convenient ocular features on the eye images prior to irradiation to detect, on-line, potential geometric mismatches. Due to this rather invasive procedure, systems for the non-invasive and quantitative monitoring of eye motion during ocular EBRT are now being investigated. Shin et al developed an optical eye tracking device for gated ocular proton therapy treatments. In this, a reference eye image corresponding to the prescribed treatment position is used to estimate eye deviations during dose delivery through image pattern-matching based on a normalized cross-correlation.

In ocular SRT treatment at the Medical University of Vienna, a single-camera device calculates horizontal and vertical eye rotations from the position of the center of the pupil and the geometrical properties of a standardized model of the human eye. Even though the above-mentioned devices have been clinically tested, the required 2D processing makes them currently unsuitable for absolute eye localization or treatment geometry verification. An alternative approach uses a 3D eye tracking device for ocular SRT based on optical coherence topography (OCT), in which eye position and orientation are estimated through anterior topography during planning CT acquisition and irradiation. A statistical eye model is then adapted to the patient specific anatomy to compute the target position. Despite being computationally inefficient and unpractical for real time monitoring, this approach is rather accurate. Wyder et al proposed a single-camera eye-tracking device by which estimates of eye position and orientation are inferred from infrared illumination and pupil recognition. However, the authors ultimately state that a multiple camera system will be necessary to improve accuracy.

The use of eye tracking techniques for eye localization in OPT entails uncertainties that needs to be addressed. Typically eye tracking stands for recognition of gaze direction, thus accurate estimate of the physical position and orientation of the eye with respect to a geometrical reference, i.e. the treatment room isocenter, is outside the scope of conventional techniques. Uncertainties in eye tracking, such as pupil imaging distortion due to optical diffraction at the cornea/air interface or corneal reflections 3D reconstruction inaccuracy, are characteristically dealt with using a calibration procedure aiming at the assessment of a set of subject-specific parameters. This procedure, though, introduce an arbitrariness that affect the measurement of actual anatomical structures. Thus, for an anatomical correct ocular referencing, these uncertainties should be considered.

The aim of the project is the development and validation of a novel eye tracking system for real-time, three-dimensional, noninvasive target localization and motion monitoring during proton therapy treatments of ocular melanomas. Such device is proposed in an effort towards the introduction a novel clinical protocol that overcomes the current requirement of tantalum markers implantation. However, the use of markers is not limited to patient positioning but extends to eye modelling and target volume definition. Therefore, the introduction into the clinical practice of the proposed system must be coupled with a different treatment planning modality resulting in a comprehensive adaptation of OPT as we know it today. The motivation for such a radical change are not necessarily related to improvement of precision and consequently treatment outcomes, given the extremely high local control rate achieved with the current clinical protocol. Indeed, a new workflow is envisioned to improve significantly treatment efficiency, safety and, mostly, patient comfort. An automatization of in-room target referencing and motion monitoring could fasten the clinical procedure ensuring a swifter experience for the patient and those applying the treatment.

The analysis of currently implemented gated protocol in ocular SRT and OPT and published experience reporting set-up errors and misalignment effect on range uncertainties in OPT was carried out to define the set of requisites for an eye tracking system aiming at accurate patient navigation and motion monitoring during irradiation. Eye orientation should be measured with accuracy lower than two degrees while patient positioning errors achieved by ETS based navigation should be lower than 0.5 mm to guarantee no detrimental effect on treatment quality with respect to the current clinical standard. In addition, a minimal frequency of 25 Hz of eye motion monitoring was identified as suitable to properly detect clinically significant involuntary eye motion.

NEW CLINICAL INDEXES FOR THE AUTOMATIC MANAGEMENT OF THE DIALYSIS TREATMENT

Domenico Vito - Supervisor: Maria Laura Costantino

End stage uremic patients number is continuously increasing involving high percentages of elderly, who are prone to comorbidities. Considering the high number of functions carried out by the kidneys, it can be easily understood how a kidney impairment could involve the whole body and in its most severe form becomes incompatible with the survival. Dialysis is the elective treatment for chronic kidneys diseases: it artificial substitutes the renal functions of removing waste and excess water from the blood. The tolerance to the treatment can vary among different individuals, also in the presence of similar prescriptions due to the peculiar patient-machine interaction. Intradialysis hypotension (IDH) is one of the main short – term hemodialysis complications, occurring in 25- 30% of cases and there are no standardized clinical protocols that provide an accurate blood pressure monitoring and the preventive. The factors involved in the onset of hypotension in patients undergoing dialysis are both due to clinical conditions (vascular or cardiac diseases, neuropathology, anemia) and treatment settings In particular the causes of hemodialysis-induced hypotension are multifactorial and related to the interaction between the patient and the dialysis procedure. Despite hypotension is still one of the most common acute dialysis complications, currently there is still a lack of clinical techniques to detect its onset during the conventional treatment. The goal of the research was to develop new methods for the evaluation of the dialysis treatment in order to enable its automatic management. Particullarly is addressed in the identification of a set indexes and algorithms for the detection of hypotension during the treatment. The study has been developed in collaboration with four clinical centres between Italy and Switzerland in the frame of the DialysIS project. The development of innovative indexes was then based on data recorded during real hemodialysis treatments. A Federated DataBase System (FDBS) approach was adopted to construct a common data repository. The storage system was built by the Dialysis Data Infrastructure (DDI), a unique multilevel standardized data structure supported by the Dialysis MATlib (DM), an embedded Matlab® library, capable to threat and manage data in the different formats collected from different centres. The Dialysis Data Infrastructure currently contains the acquisition of 1020 dialysis sessions performed on a total of 145 patients A statistical analysis was conducted on the collected data in order to find the potential risk factor related to IDH onset, both at early stages and during the treatment. A predictive index, J, in two versions J1 and J2 was defined as a weighted patient-specific combination of potential risk factors in order to predict the IDH onset at the early stage of the treatment. The indexes were also tested in their predictive ability, experimenting also different threshold for the prediction. The statistical analysis performed considering the intra-treatment period has also a multiparameter criterion, for the intra-treatment identification of IDH onset was also developed. Besides the inferential statistical analysis a machine learning approach was tested to predict IDH event from pre-dialysis conditions and considering the IDH prediction as a binary classification problem Machine learning, is a branch of artificial intelligence (AI), that focuses on finding algorithms capable of learning and/or adapting their structure, based on a set of observed data, with adaptation done by optimizing over an objective or cost function In the past couple of decades it has become a common tool in almost any task that requires information extraction from large datasets . One, of the many applications of this approach is the creation of classifiers that can separate two or more classes based on attributes measured in each subject. Machine learning techniques have raised an increasing interest in the biomedical community as they potentially offer the possibility to improve the sensitivity and/or specificity of detection and diagnosis of disease. Three different algorithm has been explored for the prediction of IDH event as result of a binary classification problem: Random Forest, Artificial Neural Networks and Support Vector Machines. The considered dataset presented unbalanced classes: the class of interest (i.e. sessions with hypotension events) was only about the 10% of the total. In order to get effective learning from the data, minority class oversampling was required. Different techniques (e.g. bootstrap, SMOTE) combinations were tested and compared. The results showed that J1 index allows predicting the 77% of the IDH events, when the threshold is set equal to 1. The use of center specific thresholds allows slightly improving index specificity and sensitivity, but does not substantially alter the results. Indeed the multiparameter criteria allows to identify the 75,56% of sessions with hypotension as defined by IDH-D, with the 24,44% of false positives. Among the tested machine learning algorithm, the SVM model balanced with high minority class SMOTE and no majority class down sampling techniques, showed the best performance and the smallest variability interval (e.g. overall accuracy: 88.26% ± 2.80%). In conclusion this work provides a set of indexes and alghorithms for the prediction of the IDH onset during the dialysis treatment. They represent a first step in the direction of a better and tailored dialysis treatment

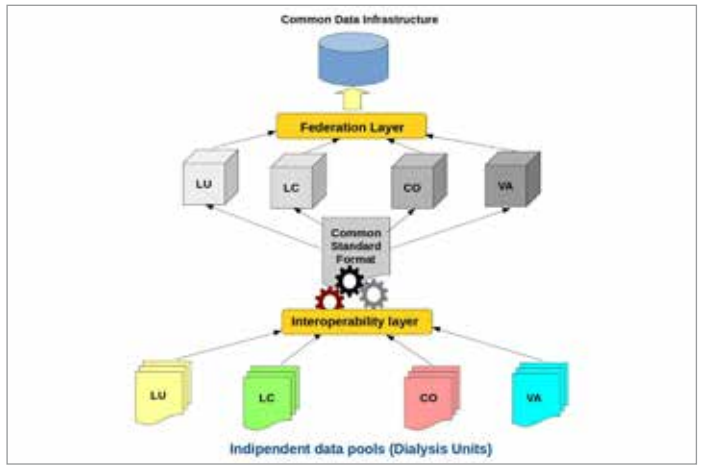

Fig. 1 - The Federate Database Infrastructure

Fig. 2 - Two IDH prediction Approaches

		ACC	PRC	RCL	AUC	f-measure
RF	Val.	77.31% ± 4.08%	73.21%	85.89%	0.833 ± 0.0029	79.01% ± 3.77%
	Ind.	81.25%	76.06%	93.10%	0.871	83.72%
ANN	Val.	80.69% ± 3.57%	78.23%	84.80%	0.869 ± 0.041	81.41% + 3.13%
	Ind.	84.82%	90.20%	79.31%	0.898	84.40%
SVM	Val.	88.26% ± 2.80%	78.23%	89.90%	0.948 ± 0.020	88.52% ± 2.66%
	Ind.	91.96%	87.93%	96.23%	0.965	91.89%

Tab. 1 - Machine Learning Algorithm Comparison