

MECHANICAL ENGINEERING | PHYSICS |  
PRESERVATION OF THE ARCHITECTURAL  
HERITAGE | STRUCTURAL, SEISMIC  
AND GEOTECHNICAL ENGINEERING |  
URBAN PLANNING, DESIGN AND  
POLICY | AEROSPACE ENGINEERING  
| ARCHITECTURAL COMPOSITION |  
ARCHITECTURE, BUILT ENVIRONMENT  
AND CONSTRUCTION ENGINEERING |  
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 | MANAGEMENT ENGINEERING  
| MATERIALS ENGINEERING | MATHEMATICAL  
MODELS AND METHODS IN ENGINEERING



Chair:  
**Prof. Andrea Aliverti**

## DOCTORAL PROGRAM IN BIOENGINEERING

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 ( $\mu$ BS Lab, DEIB), Computational Biomechanics Lab (DEIB), Biocompatibility and Cell culture Lab (BioCell, CMIC), Bioreactors Laboratory (CMIC). The Istituto di Elettronica, Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT) of the Consiglio Nazionale delle Ricerche (CNR), which is located at DEIB, represents another possible option. Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the PhD candidate training. The candidates are encouraged to carry out part of their research activities in contact with other research groups, preferably abroad through periods of at least three months spent in laboratories where the candidate can acquire further skills to develop his/her research work and thesis. Collaborations that may involve the PhD students are presently active with several national and international research and academic Institutions. Very often, the involvement of 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), Biomedical Images (2018). 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.

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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.

# COMPUTATIONAL CEREBELLAR MODELS AND THEIR EMBODIMENT IN BEHAVIORAL LOOPS TO UNDERSTAND NEURAL BASES OF MOTOR LEARNING

Alberto Antonietti - Advisors: Prof. Alessandra Pedrocchi, Claudia Casellato

Computational neural models are fundamental to explain how ensemble brain functions might emerge from elementary neuronal components. This project has been focused on the generation and tuning of cerebellar computational models, which had been gradually refined to increase their biological realism. Eventually, these computational models have been embedded within simulated and real robotic platforms and challenged in various closed loop protocols.

The models were developed as realistic Spiking Neural Networks (SNN) and have been tested in different sensorimotor tasks: Eye Blink Classical Conditioning (EBCC), Vestibulo-Ocular Reflex (VOR), movements perturbed by force fields, and motor correction. Eventually, fitting real experimental data coming from human or animal subjects. A mechanistic quantitative interpretation of the dynamic evolution of cerebellar plasticity during skill acquisition is still lacking, and computational models can be very effective to provide new insights. Thus, an extensive analysis of the relations between model parameters and behavioral outputs have been exploited. As a first step, a realistic model was developed to test neurophysiological theories,

such as the confirmation of the role of multiple plasticity sites in cerebellar learning. The simulations demonstrated that the addition of nuclear plasticity sites improved model performance and showed the different timescales of learning. We validated the model robustness in learning associative responses with different inter stimuli intervals and we have shed light on acquisition, extinction and consolidation mechanisms, associable to the different active plasticity sites.

As a second step, we used the cerebellar model to generate expectations from potential ad-hoc experiments and to generate hypotheses about the mechanisms underlying behavioral components and modifications. By manipulating the model parameters, it was possible to tune the model to fit the behavior of either healthy subjects or subjects perturbed by Transcranial Magnetic Stimulation (TMS). We have shown that a macroscopic measurement during a behavioral task can be successfully explained by using an appropriate model constructed at the microscopic level. In particular, we have been able to tune the cerebellar model against human EBCC data before and after perturbation with TMS, in two different protocols. The results supported the emerging

view that cerebellar plasticity is a dynamic and distributed process, in which cortical plasticity is more rapidly activated and drives a set of changes that reverberate onto the more slowly adapting nuclei. Changes or deficiencies occurring in one site are compensated by the others suggesting possible interventional sites for therapy and repair. The model predictions are warranted future experimental investigations, e.g. performing in vivo multi-electrode recordings of the plastic evolution of neural discharges in cortical and deep cerebellar nuclei neurons. As a third step, the neural network model was challenged in different learning paradigms, integrated with robotic platforms to the simulated "little" brain, closing the loop between action and perception. As a result, we have efficiently linked low-level cerebellar circuits with high-level functions integrating a SNN into a neurorobot operating in real-time. The main added value of learning in our neurorobot is that it succeeded in reproducing how biological systems acquire, extinguish and express knowledge of a noisy and changing world, in multiple cerebellum-driven learning tasks performed by a real robot moving in perturbed environments. The real world is

always noisier than the worst-case simulation can accomplish, and learning is actually a long-lasting experience-dependent change in behavior, which can realistically be observed only from an embodied system. This approach has the challenging potential to represent the initial building block of a more complex brain-inspired controller, into which other realistic SNNs emulating different brain structures involved in sensorimotor integration could be embedded.

During the project development, multiple features have been implemented in the model, taking inspiration from physiology, in order to increase its realism and

potentiality. The network size was increased, including geometrical information and plausible convergence/divergence ratios and plastic mechanisms. We have exploited a new SNN simulator, the NEST simulator, a popular and more advanced tool, improving the realism of the model, including new neural populations and synapses. Leveraging High-Performance Computing systems, we succeeded in generating a full reconstruction of the mouse flocculus (almost 1 million neurons and 360 million synapses) and simulating it in two learning tasks (Figure 2).

The technological platform that has been developed in this

project can lead to accelerated computational brain research since we have demonstrated the use of an effective tool-flow in the research of complex brain models. This platform showcases a paradigm for large-scale simulations that can be adapted to any other neuron modeling problems, greatly accelerating the scientific process and the development of brain research.

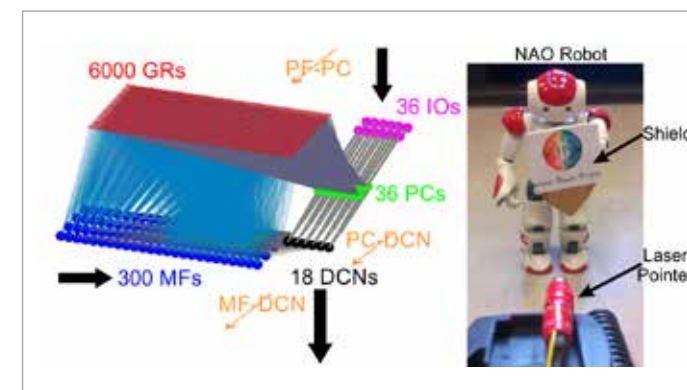


Fig. 1 - (Left) The architecture of the cerebellar SNN, the different neural populations are connected by synapses, the three plasticity sites are highlighted in orange: the cortical plasticity (PF-PC) and the nuclear plasticities (MF-DCN and PC-DCN). (Right) The robotic set-up of the Pavlovian task reproduced with NAO robot, which had to protect himself with the provided shield from the laser beam.

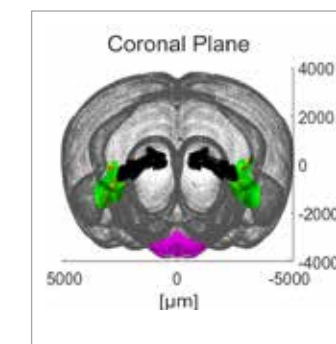


Fig. 2 - The 2D projection on the Coronal Plane of the mouse brain volume (in grey) and the 3D positions of the neurons used to reconstruct the SNN circuit of the mouse flocculus, the olivary nucleus and the deep cerebellar nuclei.

# FLUORESCENT TRANSCRIPTION FACTORS AS TOOLS TO EVALUATE THE INFLUENCE OF MECHANICAL STIMULI ON NUCLEAR IMPORT

Lucia Boeri

Advisors: Manuela Teresa Raimondi, Diego Albani, Emanuela Jacchetti

Mesenchymal stem cell (MSCs)-based therapy is a promising strategy to regenerate injured tissues and regulate immune responses. The challenges concerning the clinical application of MSCs injection are mainly related to the reduced post-transplantation cell survival and the low control of MSC fate. Recent advancements focused on MSC response by studying their mechanotransduction, that is the mechanism by which MSCs transduce environmental mechanical inputs into chemical signals. The NICHOID project started in 2015 with the goal to verify the hypothesis that MSC key mechanisms, such as differentiation, are regulated by stretched-dependent nuclear import of specific transcription factors. According to this hypothesis the nuclear pores are mechanically sensitive elements and respond to cell stretching with nuclear pore enlarging and increased nuclear import of transcription factors. The experimental validation of the NICHOID hypothesis is based on the nuclear import rate measurement of transcription factors able to promote MSC differentiation. The aim of the Ph.D. thesis was to engineer transcription factors to achieve fluorescent tools to allow the

nuclear import rate assessment in different MSC stretching conditions. To reach the goal, five fluorescent transcription factor variants were designed, purified and validated. The transcription factor experimentally selected was a promoter of myogenesis, MyoD (Myoblast determination protein). Five variants of MyoD were purified, each fused to a different fluorescent protein (probe): Tat-GFP, (-30)GFP, PAGFP, PAmCherry, mEOS3.2. Each variant of MyoD presented a peculiar characteristic: Tat-GFP-MyoD and (-30)GFP-MyoD followed specific protein transduction-based delivery procedures while MyoD-PAGFP, MyoD-PAmCherry and MyoD-mEos3.2 were sensible to photoactivation or photoconversion. The validation of the fluorescent molecules required analysis of protein localization by fluorescence confocal microscopy, nucleocytoplasmic transport measurement by fluorescence recovery after photobleaching (FRAP) method and transcription promoting activity assay by real-time PCR. The results showed that the fluorescent variant of MyoD best representing the physiology of the native MyoD is the MyoD-PAGFP. (-30)GFP-MyoD resulted a suitable tool for nucleocytoplasmic transport measurement but

real-time PCR results highlighted an inhibitory effect on MyoD functionality. Key experimental evidence of the fluorescent transcription factors was deepened with computational investigations. In particular, the (-30)GFP inhibition effect observed during the transcription promoting activity analysis was assessed by molecular dynamics (MD). MyoD-PAGFP was used as negative control in MD simulation. (-30)GFP and PAGFP 3D models were generated by homology modeling, while in the case of MyoD protein, the 3D structure of the basic helix loop helix (BHLH) DNA binding domain was the only available. The goal of MD analyses was to verify if the presence of the peculiar major negative surface charge of (-30)GFP could interact with BHLH domain of MyoD, thus altering its functionality. Considering the bias of the lacking part of the MyoD structure, they were generated four different configurations by positioning the BHLH domain of MyoD in different positions in respect to the fluorescent protein, so that the interface between the two molecules was different in every simulation. When analyzing root mean square deviation (RMSD) data and non-bonded interaction energies, results showed that (-30)GFP interacted more strongly with the monomer

of MyoD in comparison to PAGFP. Furthermore, structural analyses showed that the interactions between (-30)GFP and MyoD were mainly mediated by negative and positive residues, respectively. In conclusion, this work developed and validated several potential tools for nucleocytoplasmic transport measurement. Among the fluorescent variants of MyoD, the photoactivatable MyoD-PAGFP is the tool that best resembles the properties of the native transcription factor and it is suitable for further nuclear import measurements. Otherwise, (-30)GFP-MyoD showed great properties in terms of protein delivery and nucleo-cytoplasmic shuttling. In this variant, MD simulations suggested an interference mechanism based on a strong interaction between MyoD and (-30)GFP that could explain the inhibition of MyoD activity. Overall, this work demonstrated that, the design and the development of the fluorescent probes in an intracellular dynamics study are fundamental steps to deeply understand their possible applications.



# RESPIRHÓ: AN INERTIAL SENSOR-BASED SYSTEM FOR DAILY LIFE BREATHING MONITORING IN NEUROMUSCULAR PATIENTS

Ambra Cesareo - Advisor: Prof. Andrea Aliverti

The main function of the respiratory system is to guarantee appropriate gas exchange which involves the uptake of oxygen and release of carbon dioxide. Ventilatory control system attempts to correct deficiencies of oxygen (hypoxemia) and/or excess of carbon dioxide (hypercarbia) by increasing both tidal volume and respiratory rate, that are the main variables of breathing pattern. Breathing frequency, therefore, is per se an important informative parameter of respiratory function that may be a key predictor of adverse events. Monitoring breathing frequency, could early identify patients at risk of developing respiratory and cardiac dysfunction, with high specificity. The need for accurate, objective methods to assess and monitor breathing frequency is thus evident, inside and outside the clinic. Monitoring tools should be less invasive and obtrusive as possible, easy to use and low cost to foster the autonomous use by patients and promote the dissemination and transfer of the technology to the clinical and healthcare practice. Systems proposed in the literature for breathing pattern monitoring during daily life activities do not address these requirements and are confined to research application. An emerging approach

that could answers to the abovementioned needs is based on the measurement of chest wall movements related to breath, using inertial sensors. Assessment of breathing pattern by measuring chest wall displacement, or derived tissue changes, it is acknowledged as preferred monitoring method because does not interfere with the airways, being minimally invasive. Inertial sensors open the door to new opportunities to develop this approach in a low-cost, wearable and easy to use manner. The present thesis wants to offer new insights into breathing monitoring by using inertial sensors, trying to overcome limitations of this fields, such as high sensitivity to motion artefacts and limited validation in clinical population. A new wearable, modular and wireless device for long-term monitoring of breathing frequency has been designed, developed, implemented, tested and optimized. It is based on sensor fusion of data collected from accelerometer, gyroscope and magnetometer (Magnetic and inertial sensor Unit, MIMU) to compute complete quaternion-based orientation, and its variation over time. The device is composed by three MIMU units. Two units are placed on the thorax and on the abdomen, to take into

account the two-degree-of-freedom model of the chest wall. The third MIMU unit is used to have a local reference system, recording body (non-breathing) motion, and works as logging node. The device is integrated into an acquisition platform, in which data recorded by the units are sent to a smartphone and stored into a remote server, for telemonitoring service. Figure 1 shows the concept of the platform, describing the three sensor units. A new position-independent algorithm, to extract breathing information from orientation changes recorded with the three units has been developed and tested on healthy subjects in static and semi-static conditions, using Optoelectronic Plethysmography as reference method. A study comparing different approaches has been conducted, to identify the best method of dimension reduction allowing to move from 4-dimension problem, characterized by the four components of the orientation quaternion to a single-dimension problem, with a single signal representing the breathing signal. This study underlined as the approach based on the fusion of the four quaternion components by using Principal Component Analysis provided best estimation than approaches based on the

selection of a single quaternion component. The algorithm has been subsequently adapted to dynamic conditions (e.g. walking), allowing automatic breath-by-breath extraction of breathing pattern temporal parameters. Finally, the device and method developed have been applied and tested on clinical target population, namely Muscular Dystrophy (MD). It is characterized by progressive muscle-weakening and wasting conditions, that affect also respiratory muscles, leading to respiratory failure, the main cause of death in these patients. A pilot study to assess usability, acceptance and wearability of the device in 15 patients with Duchenne Muscular Dystrophy and Limb-girdle Muscular Dystrophy has been

approved by the Italian Minister of Health. The main aims of the study were to validate the device in static conditions, to assess its feasibility of autonomous use during daily activities and ultimately to collect information about the need of further design improvements. Data of this pilot study are reported in my thesis, representing the first available results in the literature about breathing rate assessment, obtained by using inertial-based devices in patients with respiratory muscular weakness. The pilot study on clinical population is ongoing and, when it will be finalized, it will provide information about necessary adjustments for what concerns the design of the device (e.g. dimension, shape of the housing, fixation methods...)

and analysis method; however, some conclusions can already be drawn. Preliminary data collected from the first patients enrolled, provided encouraging results considering the device comfortable, tolerable and easy to use. Validation in static conditions gave good results in terms of reliability and agreement with the reference method. Data obtained during daily activities demonstrated the feasibility of monitoring breathing rate for long periods using the device developed. The research activity described in this thesis represents a step forward the implementation of at home continuous breathing frequency monitoring in patients at high risk of developing respiratory dysfunction and failure.

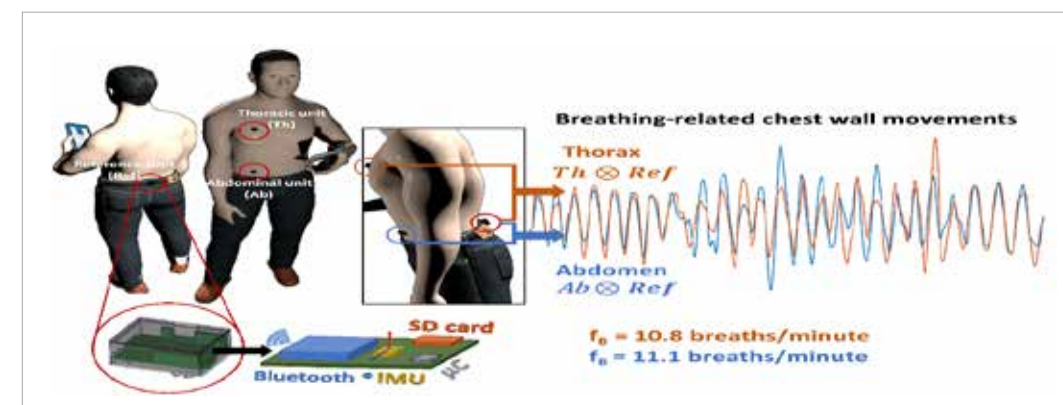


Fig.1 - Proof of concept of a wearable, wireless, modular respiratory Holter based on inertial measurement units (IMUS) for the continuous breathing pattern monitoring through the detection of chest wall breathing related movements.

## Nicola Contessi Negrini - Advisor: Prof. Silvia Farè

Adipose tissue (AT, commonly named as fat) is a loose connective tissue dedicated to energy storage, organs protection, and contributing to organism homeostasis. AT can be affected by severe pathologies, including lipodystrophies, congenital defects, trauma or resection after pathological cases (e.g., breast tumor removal) that urgently require AT restoration. Current clinical treatments mainly aim at AT volume restoration with no functional regain (i.e., synthetic prosthesis mainly in silicone), or are affected by donor-site morbidity (i.e., tissue flaps) and unpredictable long-term results (i.e., lipofilling). AT engineering offers a unique alternative to target at both tissue volume replacement and functional regain; however, strict requirements must be met to obtain an ideal AT engineering scaffold, including (i) adequate porosity, (ii) biochemical cues to allow cells adhesion, (iii) biomimetic structural properties, (iv) biodegradability, and (v) a promoted vascularization. The aim of this PhD thesis is to produce, by innovative and advanced fabrication technologies, AT scaffolds matching the requirements previously stated, by using a chemically crosslinked gelatin hydrogel investigated here for the first time for AT

regeneration.

Gelatin, a collagen derivative, was selected for its solubility in water, lower antigenic and immunogenic response compared to collagen, versatility, readily availability, and exposure of cell-binding motives and metalloproteinase target-sites for degradation. The crosslinking reaction, necessary to produce gelatin hydrogels stable at 37 °C (i.e., *in vivo* applications) and investigated for scaffolds production, is based on a Michael-type addition between gelatin and methylenebisacrylamide, the crosslinker. Gelatin concentration and reaction stoichiometry were varied to tune the hydrogels biomimetics towards different AT depots. All the produced hydrogels were stable in water at 37 °C, thus proving the efficiency of the crosslinking reaction. Different crosslinking degrees were measured by varying either gelatin concentration and/or reaction stoichiometry; the hydrogels showed different weight variations after immersion in physiological-like environment, which in turn influenced their mechanical and rheological properties. The variation of synthesis parameters allowed controlling the properties of the produced hydrogels so to achieve biomimetic properties of different AT depots (i.e., breast and heel pad AT). Moreover,

*in vitro* adhesion, proliferation and adipogenic differentiation of preadipocytes on the gelatin hydrogels were demonstrated. A strategy based on the use of sacrificial hydrogel microbeads and 3D printed structures was subsequently engineered to obtain a porous crosslinked gelatin hydrogel with a controlled vasculature, respectively. Microbeads and 3D printed strands with controlled dimension and geometry were simultaneously embedded in the gelatin hydrogel during its preparation and, at complete gelatin crosslinking, the sacrificial structures were removed by an optimized procedure. The so-produced scaffolds showed ideal properties in terms of porosity (200 – 400  $\mu\text{m}$  pore size), mechanical compressive response ( $E = 3 \text{ kPa}$ ), and enzymatic degradability. Moreover, the obtained hollow channels allowed fluids flow (i.e., water and blood, tested *ex vivo*) and cells adhesion to the hollow channels walls, promising aspects for a promoted vascularisation. Finally, *in vitro* tests proved the ability of the produced scaffolds in supporting human mesenchymal stem cells adhesion, proliferation, and adipogenic differentiation (figure 1).

To achieve a controlled

macroscopic shape with a defined porous structure, the crosslinked gelatin hydrogel was also used as ink and 3D printed by an optimized procedure to obtain 3D printed scaffolds for AT engineering. The rheological properties of the gelatin solution were investigated during the crosslinking and a printability time window (i.e.,  $G' < G''$ ) was identified. The gelatin solution was printed on a substrate by keeping the cartridge temperature at 35 °C, after printing parameters optimization (figure 2). The printed hydrogels were crosslinked at an optimized temperature and the obtained structures showed patterned geometry and macroscopic shape reproducing the CAD design used for the printing. The printed hydrogels were stable at 37 °C and biomimetic AT mechanical properties were achieved; moreover, printed hydrogels maintained their shape during *in vitro* cells cultures and sustained preadipocytes *in vitro* adhesion, proliferation and adipogenic differentiation.

In conclusion, this thesis describes, for the first time, the use of a chemically crosslinked hydrogels processed by advanced fabrication technologies useful for AT engineering. This hydrogel is a versatile material that

can be fabricated by different techniques that allow producing scaffolds successfully matching AT engineering requirements.

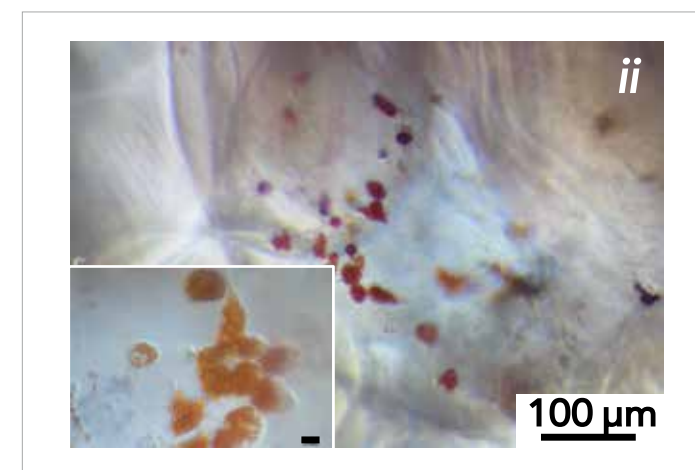


Fig. 1 - Oil Red O staining of human mesenchymal stem cells cultured on porous gelatin hydrogels

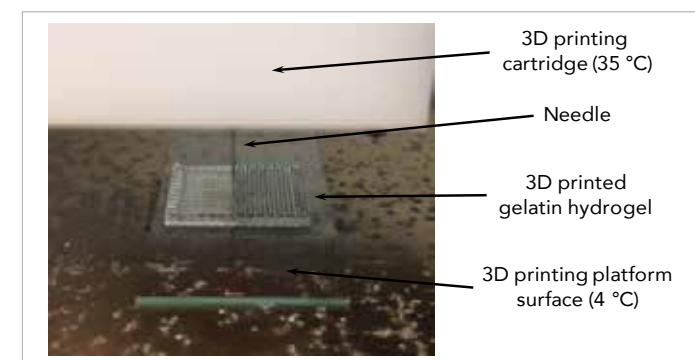


Fig. 2 - Optimized 3D printing process of chemically crosslinked gelatin hydrogels

## Beatrice De Maria

### Advisors: Prof. Sergio Cerutti, Prof. Alberto Porta

#### Introduction

The baroreflex is a complex physiological mechanism aimed at the maintenance of the arterial pressure (AP) homeostasis, exploiting different branches. The cardiac BR (cBR) branch reacts to AP changes modulating the heart period (HP), while the sympathetic BR (sBR) one regulating the sympathetic activity. The cBR is commonly characterized by computing the cBR sensitivity (cBRS) defined as the amplitude of the HP changes in response to a unit variation of AP, while the sBR by computing the sBR sensitivity (sBRS) defined as the amplitude of the MSNA changes in response to a unit variation of DAP. As a result of its inner functioning, BR reacts both to AP increases and decreases and the eventual different response of the BR to AP positive and negative variations is referred to as the BR asymmetry.

#### Aim

The purpose of this thesis is to propose the assessment of the asymmetry of BR by avoiding the use of pharmacological intervention but exploiting two analytical methods based on the spontaneous variability: the sequence (SEQ) method and the bivariate phase rectified signal averaging (PRSA) one. The BR asymmetry will be evaluated

over both cBR and sBR branches. Consequently, the first aim of this doctoral thesis is to adapt the SEQ and bivariate PRSA methods for the assessment of cBR and sBR asymmetries.

#### Experimental protocols

Three different experimental protocols were analysed: i) 12 young healthy subjects performing incremental tilt, to verify the effect of an orthostatic challenge (TILT) on cBR and sBR asymmetries; ii) 100 healthy subjects characterized by different age (20 subjects for each decade, from 21 to 70 yrs) performing active standing, to assess the effect of aging on cBR asymmetry; iii) 107 amyotrophic lateral sclerosis (ALS) patients and 44 age-matched healthy controls performing tilt, to assess the effect of a pathological condition, here ALS, on sBR and cBR asymmetries.

#### Results

The most important findings of this doctoral thesis are: i) the possibility to exploit the bivariate PRSA method to evaluate both the cBR and sBR asymmetries based on the spontaneous variability; ii) the SEQ and PRSA methods cannot be considered equivalent in the assessment of the BR asymmetry; iii) cBRS markers are modified by postural challenges and ALS; iv) sBRS markers are not

affected by postural challenge and ALS; v) when assessed from spontaneous variations the asymmetric behaviour of sBR seems to be more evident than that of the cBR in young healthy subjects and this conclusion does not hold in the ALS patients; vi) the asymmetry of the cBR is linked to the HP asymmetry; vii) in ALS patients the sBR is more impaired than the cBR, but the cBR and sBR asymmetry indexes are uncorrelated with patients' clinical features; viii) the HP asymmetry is linked to the functional status in ALS patients.

#### Conclusions

In this doctoral thesis, the PRSA method was successfully adapted to the separated computation of cBRS by considering positive and negative AP variations, both in healthy subjects and in ALS patients, and successfully extended for the characterization of the sBR. As to cBR functioning, in all the considered populations, no significantly relevant differences between cBRS estimates derived by positive and negative AP variations were detected. Conversely, in young healthy subjects the sBR exhibited a significant asymmetry with the same absolute changes of AP producing more important changes of sympathetic burst

rate in response to AP decreases than to AP increases. It can be concluded that when the cBR and sBR characterization is performed on the basis of the spontaneous variability, the asymmetry seemed to be a peculiar aspect of the sBR, but not of the cBR. From a methodological point of view, while the SEQ method seems to be more powerful for the quantification of the asymmetry of the BR branches compared to PRSA, the opposite conclusion holds when the characterization of BRS, regardless of the sign of the AP variations, is the focus. Therefore, this thesis recommends the use of SEQ method for BR asymmetry evaluation and the PRSA method for the traditional computation of BRS especially in pathological populations. Remarkably, this thesis suggests that the cBR asymmetry plays a role in producing the HP variability asymmetry. Indeed, although no significant difference was detected between contribution given by positive and negative AP variations to cBRS, the cBR asymmetry indexes were correlated to HP asymmetry ones, suggesting that the small non-significant difference between them contribute nevertheless to the HP asymmetry. However, the BR asymmetry explained only partially the HP variability

asymmetry given that, while the HP variability asymmetry was progressively reduced with age, the same trend was much weaker when the BR asymmetry markers were considered. As to the characterization of ALS this thesis confirmed that cBRS was lower in ALS patients but the response to TILT was preserved. Conversely, sBR appeared to be much more impaired than cBR given that sBR was not affected by TILT and sBR asymmetry was lost. However, these results need a check after increasing the size of the group of healthy controls. The few significant correlations of the BR parameters and asymmetry makers with the clinical status of the ALS patients stresses the need to reduce the heterogeneity of the ALS population by classifying more homogenous subgroups.

# DEVELOPMENT, CHARACTERIZATION AND FEASIBILITY OF INDUSTRIALIZATION OF A NOVEL HYBRID SEMI-DEGRADABLE VASCULAR ACCESS FOR HAEMODIALYSIS

Sebastiao N. Dentinho van Uden

Advisors: Prof. Alberto C. L. Redaelli, Stefania A. Riboldi

Early-stage renal disease patients desperately need haemodialysis to compensate the loss of natural blood filtration and maintain system homeostasis. However, for decades vascular accesses, required for haemodialysis, have been the bottleneck of this procedure, limiting the kidney transplant (3.7% chance a year) wait time to an average of 1000 days.

When possible, arteriovenous fistula is the choice to create a vascular access for long-term haemodialysis. Although, functional fistulae have the highest success rates in the long-term due to its purely native tissue composition, two thirds never reach the maturation point to allow haemodialysis – failing in the short-term. At this point synthetic grafts fill the gap, providing immediate access (24-72h after minor surgery) – early cannulation. However, being fully composed of synthetic bioinert material they are not able to biointegrate or match the compliance of adjacent anastomosed vessels, eventually failing in the long-term. Following on what fails with fistulae and synthetic grafts, it is clear how both have complementary advantages (and disadvantages). Therefore, this work envisioned an ideal vascular

access approach that behaves as a graft in the short-term and as a fistula in the long-run. This way providing the benefits of a readily available and predictable graft for early cannulation, which slowly converts into native vascular tissue; adapting and evolving according to local haemodynamics in a similar fashion as the artery and vein connected by the vascular access.

In this work, principles of *in situ* tissue engineering (Figure 1) are followed, using electrospinning, to achieve a vascular graft that performs as a bioactive scaffold, hosting the colonisation of vascular cells that trigger tissue development. Enzymes released during this process degrade most of the scaffold, allowing progressive and controlled remodelling from scaffold to vascular tissue. However, an elastic artificial bioinert mesh remains embedded in the tissue, ensuring

optimal immediate recovery from haemodialysis punctures and continuously intermediating haemodynamic divergences between artery and vein. Silk fibroin and polyurethane were the chosen materials to develop this hybrid semi-degradable vascular graft due to their respective bioactivity and elasticity. In this sense, the first milestone was to achieve an electrospun blend of these two materials – Silkothane<sup>®</sup> material – to serve as the core structure of the graft. Physicochemical analyses demonstrated the maintenance of the characteristic features of fibroin and polyurethane upon solubilisation, blending, electrospinning and post-processing with ethanol or methanol. Envisioning their possible application as semi-degradable substrates for haemodialysis, tubular meshes were further characterized,

showing sub-micrometric fibrous morphologies, tuneable mechanical properties, permeability before and after puncture in the same order of magnitude as commercial grafts currently used in the clinics. The development of the Silkothane<sup>®</sup> graft followed as a subsequent milestone to reach. Considering the demonstrated haemocompatible and cell adhesive properties of silk fibroin, the graft design concerned three electrospun concentric layers (Figure 2). Thus, composed of a thick core layer of Silkothane<sup>®</sup> material in between two thin layers of pure electrospun fibroin, providing a continuous presence of this cell adherent material across the Silkothane<sup>®</sup> graft's entire structure. The full three-layered graft, influenced by the polyurethane presence, ensured mechanical properties that are a determinant

factor for the success of a vascular access (e.g., vein-graft compliance matching). The Silkothane<sup>®</sup> graft demonstrated early cannulation potential in line with self-sealing commercial synthetic grafts, and a degradability driven by enzymatic activity. Moreover, the fibroin-only layers and extracellular matrix-like morphology, presented by the graft, revealed to be crucial in providing a non-haemolytic character, long clotting time, and favourable adhesion of human umbilical vein endothelial cells with increasing viability after 3 and 7 days. Accordingly, the proposed approach represents a promising step forward towards an *in situ* tissue engineering vascular access and its potential for vein-graft anastomosis stability, early cannulation, and biointegration. Finally, to ensure industrial feasibility of the Silkothane<sup>®</sup> graft, a pilot plant electrospinning system able to scale up

manufacturing, while increasing quality, was developed for clinical validation and initial commercial application. Results from directly comparing the current and the upgraded electrospinning systems revealed a 5-fold drop in cost per Silkothane<sup>®</sup> graft, placing it well below average production cost per commercial synthetic grafts, at a rate of six 30 cm-long grafts fabricated per day. In summary, the promising findings achieved in this thesis provide the means to fabricate a pioneering *in situ* tissue engineering vascular access, addressing all needs from the commercial, industrial and – potentially – clinical perspectives, while aiming to finally eliminate the half-century old haemodialysis bottleneck, which is failing vascular accesses.

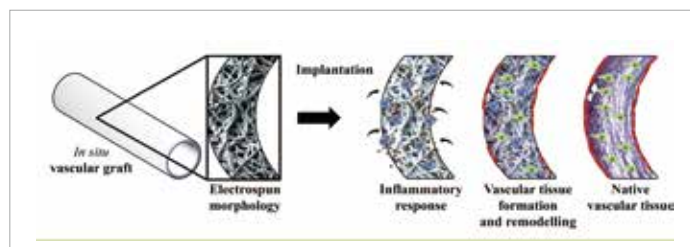


Fig. 1 - Schematic representation of *in situ* tissue engineering approach upon implantation of a vascular graft.



Fig. 2 - Silkothane<sup>®</sup> graft and respective cross-section depicting the three-layered structure.



# DESIGN AND PROTOTYPING OF A NOVEL BIODEGRADABLE MAGNESIUM-BASED DEVICE FOR INTRAVITREAL RELEASE OF ANTI-VEGF

Marco Ferroni - Advisor: Federica Boschetti

Nowadays, fully biocompatible, biodegradable and intravitreal drug delivery system, with sustained-release of anti-VEGF drugs, would be the ideal alternative to the current gold-standard intravitreal injections for the treatment of exudative age-related macular degeneration. In fact, several negative aspects are associated to this treatment strategy, such as systemic/intraocular complications, infection risks, and particularly low patient compliance and expensive therapy on National Health Systems. In this project a novel fully biodegradable and injectable magnesium-based device was designed and a first prototype was developed. Considering the absence of drug delivery systems for the treatment of the age-related macular degeneration on the market, the design of such a device has the main clinical relevance to overcome the limitations associated to the current clinical practice. In order to develop a strong and novel method that aims to the design and development of an ideal drug release device, a classic engineering design process was proposed and developed. The implementation of a novel combined computational fluid-dynamic model of the ocular posterior chamber was able to

show the influence of analytical configurations of the ocular saccadic movements and permeation characterization of surrounding tissues on fluid flow inside the vitreous body. The computational fluid-dynamic model was extended to the study of drug delivery mechanisms when a gold-standard amount of inhibitors of the vascular endothelial growth factors is injected intravitreally. The novelty in the research field in combining analytical saccadic movements and permeation characterization of surrounding tissues, in addition to the experimental evaluation of scleral hydraulic conductivity, led to computational results that confirmed the rapid enhancement to anti-VEGF distribution due to

saccades in the vitreous cavity.

Moreover, the implementation of such a computational model posed the bases for the comprehension of the fluid-dynamic conditions for the design of the drug delivery device. An injection of a hypothetical device was simulated, and the fluid-induced shear stress field on the device itself in contact with the liquefied vitreous was studied, comparing computational fluid-dynamic with fluid-structure interaction approaches. The computed fluid-induced shear stress distribution was evaluated and clearly suggested the possibility to have a consequent uniform corrosion in the majority

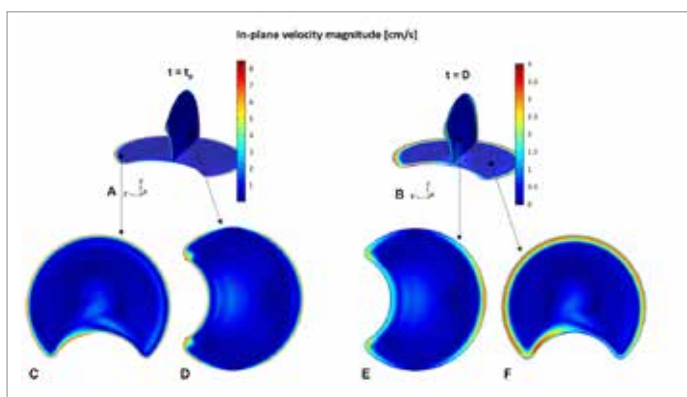


Fig. 1 - In-plane velocity magnitude in a 3D view at two time instants (A) and (B) of the combined CFD model in presence of saccades. Zoom of the equatorial (C-F) and vertical (D-E) planes of both the 3D views.

of the device, once injected in the vitreous cavity. Two stressful simulated conditions were chosen as representative of the fluid-dynamic impact of the ophthalmological milieu on the device for the analysis of biocorrosion phenomena and compared to a static control. The main body of the project led to the characterization of the magnesium, chosen as very promising biomaterial to be used as ideal platform for drug release in the ocular environment, focusing on its corrosion mechanism and rate, induced by the shear stress action. Firstly, a further computational fluid-dynamic model based on a sparse non-linear optimizer optimization method was used to design the experimental conditions in order to recreate the shear stress fields computed previously on manufactured magnesium samples. After performing ad hoc corrosion tests, morphological and profilometric maps of the free-eroded surfaces of magnesium samples were acquired by using scanning electron microscopy and confocal laser scanning microscopy to evaluate the corrosion mechanisms. The magnesium corrosion rates were evaluated (1.9, 2.7 and 3.435  $\mu\text{m}/\text{day}$ ) in three fluid-induced shear stress conditions and coupled to the current clinical timing between two consecutive injections, obtaining the prospective magnesium thickness of the final layer (57, 81 and 103  $\mu\text{m}$ ).

The reported results highlighted the great potentialities in using magnesium as reference

biomaterial for the design of drug delivery systems in the ocular field and, in addition to the implemented method, represented a novelty of relevance in the research field and for the clinical application. Noted the corrosion behavior of magnesium in the vitreous cavity, a numerical campaign was developed by using numerical analysis based on finite element method in order to define the peculiar corrosion of the magnesium layer and, consequently, the final design of the device. Then, a numerical investigation of anti-VEGF release was performed. Bevacizumab (Avastin®) was used as clinical reference drug. After the monthly layer corrosion, bevacizumab was considered as 95% uptaken after 236 seconds of continuous ocular motions. The proposed corrosion model was able to simulate more complex corrosion conditions, related to an ocular non-homogeneous shear stress field acting on the device; moreover, its coupling with the bevacizumab release model represented a perspective pre-clinical tool for the

analysis of the device performance and functioning in terms of corrosion times and anti-VEGF release. Finally, a Mg-Nd-Zn-Zr (denoted as JDBM) alloy up-scaled prototypes were manufactured for the future in-vitro experimental campaign to prove the concept, focusing on the anti-VEGF drug stability in presence of magnesium and on the effective kinetics of drug release from the prototype. The presented and developed project was registered as patent application.

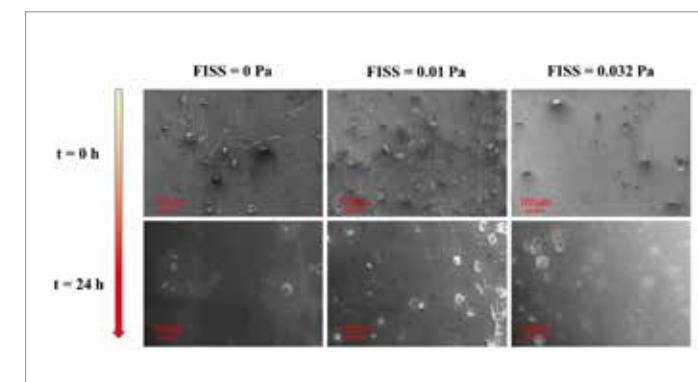


Figure 2. 24 hour time-scale analysis of the sample morphology: localizations are present before testing them ( $t = 0$ ). After 24 hours the fluid flow oxidized the magnesium localizations and then erode the corrosion products, making the eroded surface as flat.

## INNOVATIVE STRATEGIES TO CONTROL CELL FATE AND GENE DELIVERY BEHAVIOUR

Elisa Giupponi - Advisors: Gabriele Candiani, Lina Altomare

It is common knowledge that cells are naturally exposed to a multitude of stimuli in their surroundings that actively participate in determining the *in vivo* cell behaviour and fate. The control of cell behaviour and fate *in vitro* is possible through the design of biomaterials and tools with specific properties able to sustain and regulate cell adhesion, proliferation and even differentiation. Science is making great efforts to recapitulate the complex scenario of the cellular microenvironment to study cell behaviour and fate, and to this aim, several approaches such as surface modifications with topographical cues and/or chemical functionalities, and gene delivery, can be adopted. The main objective of the present thesis is the development of innovative strategies to control cell behaviour and fate: i) sol-gel chemistry has been applied for the functionalization of glass surfaces with different chemistries and variable amounts of thiols to study these groups as mediators of cell adhesion and functions; ii) a microfluidic platform was validated to trap, culture and transfect small cell populations, and for the fast screening of transfectants performances. Several strategies have been adopted for the modification

of surfaces, but the chemical functionalization has attracted more and more attention over the last decades, since it can drive and regulate the first interfacial interaction between the material and the cells. In this context, novel thiol-enriched surfaces have been developed by using the well-known sol-gel chemistry (Chapter 2), to investigate the possibility to immobilize cells exclusively by disulphide bond formation between the exofacial protein thiols (EPTs) and thiols immobilized onto the surfaces. Two different alkoxides, tetraethyl orthosilicate (TEOS) and n-propyl trimethoxysilane (CH3), each with a variable amount of (3-mercaptopropyl) trimethoxysilane (MSH), have been used for the synthesis of sols that have been deposited onto glass surfaces by means of dip-coating process. A physico-chemical characterization was carried out, followed by the preliminary biological validation. The resulting surfaces showed variable wettability, roughness and coating thickness according to their chemistry and thiol content, that depended on the recipe of the starting solutions. Moreover, the surfaces have been tested as platforms for the selective thiol-mediated cell adhesion, by using HeLa cell line. We observed that

the higher was the thiol content onto the surfaces, the higher was the number of cells retained. This result ascertained that EPTs actively participated in cell adhesion and they bound cells to thiol-presenting surfaces by means of disulphide bond formation. Based on these preliminary but appealing results, thiols were explored more in-depth as regulators of cell behaviour and functions (Chapter 3). Sol-gel spin-coating was used for the synthesis of thin and homogeneous coatings. We focused on the study of thiols role in mediating the cell response to the material by using MC3T3-E1 pre-osteoblasts. Thiol-functionalized surfaces turned out to affect cell adhesion, cytoskeleton organization, focal adhesions (FAs) formation and cell differentiation. Summarizing, we demonstrated that thiols activated the Rho GTPases pathways which are responsible for cytoskeleton and FAs assembly, as well as for lamellipodia and filopodia formation. By increasing the thiol content onto the surfaces, such effects raised, confirming that thiols were fundamental players in cell adhesion mechanisms. Finally, TEOS-based surfaces demonstrated to affect cell differentiation, by promoting osteogenesis, while a very light effect was observed for CH3-based

surfaces.

The grail of gene delivery is the development of safe and effective gene delivery vectors, and, in this scenario, there is the urgent need for the assessment of transfection efficiency and cytotoxicity in a fast and reliable manner. In this scenario, an easy-to-use microfluidic platform for the quantitative assessment of cell trapping, culture and transfection, and for the high-throughput screening of gene delivery vectors, has been validated and characterized. The device was endowed with a serial dilution generator (SDG) for the generation of a linear dilution of gene delivery particles and a downstream culture area for the trapping and culture of small groups of cells. The device allowed trapping roughly 10 cells per chamber and displayed enough room for exponential cell growth during the whole culture period. Moreover, the platform proved to be a reliable, very useful tool for the high-throughput analysis of transfection in a miniaturized fashion. The developed device allowed the screening and the optimization of the performances of gene delivery vectors through the selection of the best transfection conditions among five different transfectants doses generated simultaneously by the

SDG.

Overall, we present: i) the validation of thiol-functionalized surfaces as platforms for selective cell adhesion and the control of cell behaviour and differentiation; ii) the validation and characterization of a microfluidic device for cell culture and the screening of transfectants performances. These issues are aimed at designing a future device where thiol-functionalized surfaces are adopted as substrates of the microfluidic platform, in order to combine the fine control of cell adhesion and behaviour, with gene delivery studies.

# MULTISCALE AND MULTIPHYSICS METHODOLOGIES FOR BLOOD OXYGENATORS DESIGN

Ricardo Gómez Bardón

Advisors: Gabriele Dubini, Giancarlo Pennati

## Introduction

Blood oxygenators act as an extracorporeal artificial lung during certain types of cardiac surgery and intensive care therapies, where it is required to support or completely replace the functioning of the patient's lungs. Their operating principle consists in oxygen diffusion to blood through a semipermeable membrane, typically arranged in a set of hollow fibres. These fibres carry gas inside (intraluminal flow), while blood flows externally around them (extraluminal flow). Several engineering tools are used by the medical industry during oxygenators design, including experimental and computational techniques. Broadly speaking, the information gathered from these tools characterizes the blood flow field and temperature across the device, the oxygen transport through the blood stream, the structural integrity of the different components and the associated risk of blood damage during use. The scope of this research is to develop advanced methodologies for an optimized design of blood oxygenators. Some of the traditional assumptions adopted during their analysis will be revised, applying a different perspective to handle some of the problems associated with their development. New approaches will provide a better understanding of their functioning, being the ultimate goal to apply these methods in future designs

with optimized efficiency.

This thesis is organized on a multiscale and multiphysics perspective, combining computational fluid dynamics (CFD) and experimental microfluidics. After an introduction to the research problem and our goals (chapter I) and a historical background (chapter II), we will focus on different physical phenomena relevant to blood oxygenators. Chapters III and IV cover fluid dynamics (blood flowing around the fibers), multiphase effects of blood (cells suspended in plasma) and mass transport ( $O_2$  diffusion through blood). Moreover, chapter V investigates heat exchange (blood temperature regulation and water condensation on the gas phase). Each of them will be considered at different levels, from the device as a whole (tens of centimeters) to the red blood cells (RBC, a few microns). Finally, general conclusions and possible applications for future designs will be discussed in chapter VI. The main contributions of this work to the state-of-the-art in blood oxygenators are an innovative CFD modelling approach for an accurate description of the fibre bundle; an experimental investigation of the heterogeneous distribution of RBC around the oxygenating fibres, and a predictive model for the risk of condensation during use.

## Research goals

During the last 70 years, a substantial evolution of extracorporeal blood

oxygenating devices has been achieved thanks to the combined efforts of medical institutions, engineering companies and research centers. Over the last decade, survival rates above 99% have been reached<sup>1</sup>, with an estimated use above 650,000 patients worldwide per year<sup>2</sup>. Although different technologies are available in the market for blood oxygenation, here we will focus on the most widely used. This consists on a set (often referred to as *bundle*) of hollow fibres made of a microporous membrane. A gas mixture flows inside the fibres, while blood circulates outside them. Thanks to the submicron-sized pores inside the fibre membrane,  $O_2$  and  $CO_2$  can be exchanged across both sides (a detailed description will be given in Chapter II). This way, the gaseous composition of blood is regulated in an equivalent way to what occurs inside the human lungs, with the main difference that the oxygenation occurs *outside* the patient's body (being blood oxygenators non-implantable devices). Typically, a small heat exchanger is included in the device to control the blood temperature during its use. Despite this great success, some considerable challenges are still posed by the oxygenators complex structure, the wide range of length scales involved or the different physical phenomena governing their efficiency. Among the various issues

to be considered, we could mention the integration of the oxygenating bundle and heat exchanger, or the configuration of feed and discharge sections and the limitations imposed by progressive volume reductions; scaling from the red blood cells (RBC, of a few microns) to the overall device dimensions (dozens of centimeters); coupling of blood flow, hemorheological response and biocompatibility; temperature gradients and  $O_2$  and  $CO_2$  diffusion; counterbalance of global performance, production cost and usability... As will be seen in Chapter II, the design of blood oxygenators integrates a wide range of engineering and medical factors, many of which still demand a finer analysis and improved solutions. In this sense, this work is focused on the development of efficient methods for the analysis of blood oxygenators, aiming to introduce useful tools with an industrial application during their design process. For this, a number of simplifications frequently adopted to describe oxygenators behavior will be reconsidered, in pursuit of a further insight into the mechanisms underlying their performance. A combined approach of numerical techniques (Computational Fluid Dynamics, or CFD simulations) and experimental work (microfluidic devices) will be applied to the following topics:

- the CFD modelling of the oxygenating fibre bundle: to what extent is the traditional approach (porous media model) accurate under certain configurations of modern devices?
- the treatment of blood as a homogeneous fluid: does its multiphase nature (as suspension of RBC in plasma), have any influence?

- the handling of water condensation on the gas side (one of the most common issues during oxygenators clinical use): how is it generated, and how can we prevent it?

## Work structure

The three arguments listed above will be developed in the following chapters following a multiscale/multiphysics structure, organized attending to the effects of each physical phenomenon at different geometric scales. This approach aims to reveal their interconnections towards an overall view of the performance of the oxygenating device. Chapter II will present a historical background on the evolution of blood oxygenators, together with a brief outline of the most common engineering tools used in the design of modern devices.

Chapter III starts by describing in greater detail the traditional computational approaches for representing the blood fluid dynamics inside oxygenating bundles. Their motivations and the needs for their simplifications will be discussed, and a novel strategy for setting up more accurate models in an efficient way will be described. The entire process of model generation and analysis will be presented, illustrating the advantages of its application for the design of new devices. Limitations of classical models will be assessed by comparison with the suggested alternative, considering the most common arrangements in modern oxygenating bundles. Special attention will be given to the different effects at the fibre level (flow micro-patterns) and along the different regions of the device (macro-level perfusion of the oxygenating bundle). Finally, an extension of the benefits from the strategy proposed to oxygen

transport modelling will be presented. Chapter IV moves from the geometry analysis to the fluid characterization, focusing on the description of blood as a multiphase suspension of RBC in plasma. For this, the first part of the chapter will describe experimental work with microfluidic channels inspired in the oxygenating bundles. Blood flow (in particular, RBC patterns) will be analyzed in setups replicating the fluid dynamic conditions equivalent to real clinical use. Again, the effects at different characteristic lengths (single fibre and entire microchannel) will receive special attention. Complementary, the second part will describe a CFD multiphase blood approach, replicating the experimental results. This method will be then coupled with the strategy for describing fibre bundles from Chapter III, exploring its usefulness during the analysis of new design configurations. Finally, the implications of the multiphase nature of blood in a more accurate modelling of oxygen transport will be described. Chapter V presents a detailed description of water condensation on the gas side of blood oxygenators during their use, considering the various mechanisms (air humidification, heat transfer and change of phase) governing the process through different sections of the device. Finally, the influence of the suggested method for fibre bundle numerical modelling from Chapter III will be discussed, evaluating its usefulness for design purposes in comparison with traditional methods. Chapter VI will conclude by summarizing the findings from the previous sections, trying to give a general perspective of their implications in possible optimizations of future designs.

# INVESTIGATION OF HEART VALVE TRANSCATHETER THERAPIES – FROM A PLATFORM TO A NEW THERAPY CONCEPT

Michal Jaworek

Advisors: Riccardo Vismara, Alberto Redaelli

## Background

The aging of society and associated comorbidities are major stimulators for the spread of valvular transcatheter therapies. This sets new challenges for clinicians, researchers and device developers.

The need for new valvular treatments or improvement of existing ones is related to poor clinical outcomes of current state-of-the-art therapies, especially in case of functional mitral and tricuspid regurgitation, which showed high pathology recurrence rate in long-term. Moreover, tricuspid regurgitation treatment specifically has been neglected surgically until recent years and the available interventional options are limited.

The quest for new, more durable and less invasive therapies can be assisted with realistic preclinical evaluation. Reliable experimental platforms tailored for transcatheter therapies and featuring models of valvular pathologies could help in accelerating the development of new therapies. Moreover, a possibility for hand-on high-fidelity training in transcatheter device implantation could help to potentially reduce the occurrence of procedural errors and improve the learning curve.

The aim of this dissertation was to facilitate current transformations in valvular therapies by development of novel passive beating heart platforms featuring models of valvular pathologies and their application in studying new transcatheter therapies and clinicians' hand-on training.

## Experimental toolbox tailored for transcatheter therapies

Experimental models of functional tricuspid regurgitation and functional mitral regurgitation were developed and incorporated into passive beating heart platforms.

A mock circulation loop of pulmonary circulation was designed, manufactured and assessed. The platform (Fig. 1) housed whole right porcine heart passively actuated under pulsatile flow conditions. The incorporated experimental model of functional tricuspid regurgitation was reversible, easily manageable and yielded stable hemodynamic conditions over simulated typical procedure time. We tackled the challenges related with the management of cyclically pressurized compliant-wall right ventricle and control of the tricuspid regurgitation state in an experimental setup. The developed platform enables systematic preclinical assessment

of novel treatments under repeatable and controllable setting, which is not possible to be obtained in real clinical scenario. An experimental approach to induce functional mitral regurgitation conditions in a cyclically actuated healthy porcine left ventricle was explored. The proposed method was based on mechanical dilation of mitral annulus and displacement of left ventricular papillary muscles using *ad-hoc* designed and 3D-printed devices. This strategy provided a realistic, repeatable and reversible ex-vivo pathological model, featuring lesions clinically associated with the pathology in terms of hemodynamics, valve morphology and kinematics. It is the first experimental approach which reproduced actual main determinants of functional mitral regurgitation in whole heart

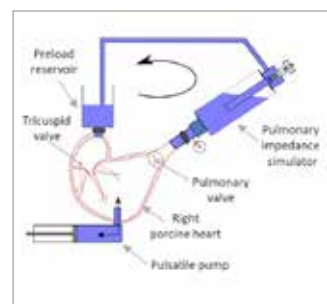


Fig. 1 - Passive beating platform scheme.

sample under pulsatile flow conditions.

## Novel treatments assessment

The tricuspid regurgitation is conventionally treated at valve-level only. However, papillary muscle displacement is, next to annular dilation, the main determinant of the tricuspid regurgitation. In this work, right ventricular papillary muscle approximation was comprehensively assessed in an *ex-vivo* model of functional tricuspid regurgitation under tight control of the direction and level of the approximation with the support of computational 3D morphology reconstructions from volumetric echocardiographic data. The treatment caused a significant reduction of the tricuspid regurgitation, tricuspid valve tenting volume and dimensions of tricuspid annulus (Fig. 2). Moreover, low baseline cardiac output and low baseline dimensions of the tricuspid annulus were identified as the predictors of treatment success. In a follow-up study we demonstrated that the greatest reductions in regurgitation and morphological anomalies of the tricuspid valve were obtained after the concomitant treatment (at valvular and subvalvular level) addressing directly both determinants of the pathology. Next, we investigated the hemodynamic effects of a new design of a transcatheter edge-to-edge device for mitral and tricuspid insufficiency, featuring a long-arms clip. A standard-length clip is widely used commercially. The long-arm clip could ease grasping procedure, however the

impact on the hemodynamics was unknown. In the experimental models of mitral and tricuspid regurgitation, the long-arm clip treatment improved overall hemodynamic conditions without inducing significant increase of transvalvular pressure drop across mitral and tricuspid valve. We demonstrated that tricuspid valve clip-based therapy was successful when septal leaflet was grasped along with either anterior or posterior one. It confirmed initial clinical observations and experimental observations with standard-length clip. Moreover, it was found that left atrial pressure monitoring could be used as an additional indicator of procedure success.

## Hands-on training

The experimental platform was

adapted for hands-on clinicians' training in transcatheter therapies. The training-oriented platform was employed for various training purposes including (i) facilitating the definition of training strategies in transcatheter edge-to-edge treatment of tricuspid regurgitation and providing visual data for training materials, (ii) serving as demonstrative and training tool during cardiological congresses across Europe in transcatheter therapies for mitral, tricuspid and aortic valve. This work demonstrated a possible spin-off application of platforms initially intended for research purposes.

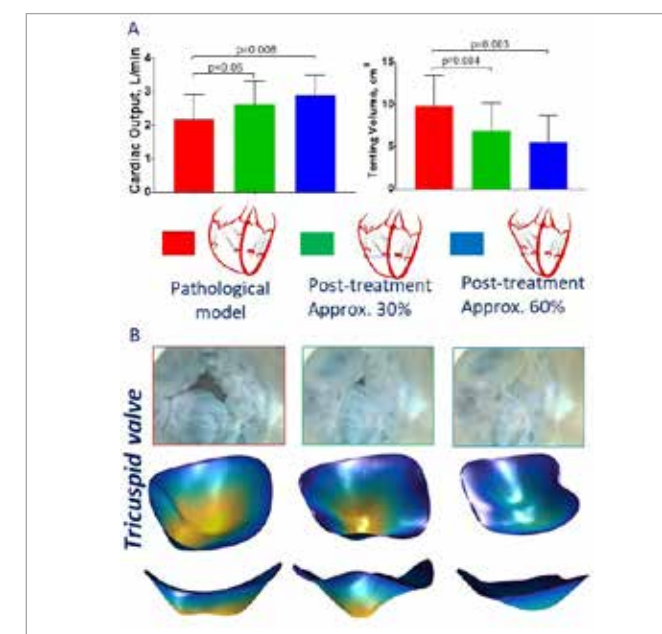


Fig. 2 - Assessment of right ventricular papillary muscle approximation concept for treatment of functional tricuspid regurgitation in a beating heart platform. A: hemodynamic and morphological changes following the treatment, B: images of tricuspid valve in peak systole from fiberoptic and 3D computational reconstruction from echocardiographic data.



# CHARACTERIZATION OF AUTONOMIC AND CARDIORESPIRATORY CONTROL IN NEWBORN POPULATIONS AT RISK FOR SUDDEN INFANT DEATH SYNDROME

**Maristella Lucchini - Advisor: Prof. Maria Gabriella Signorini**

**Co-Advisor: Prof. William P. Fifer**

Sudden Infant Death Syndrome (SIDS) is defined as a sudden and unexplained death of an infant younger than 1 year of age. Although infrequent, SIDS is still the most common cause of infant death between 1 month and 1 year in developed countries. Thanks to epidemiological, animal and pathophysiological studies, possible risk factors and mechanisms leading to this death are more understood, nonetheless number of deaths has reached a plateau in the last decade and no reliable quantitative tool to assess risk exists. The purpose of this PhD thesis is to employ novel signal processing methodologies to accurately estimate the effect of several risk factors on the autonomic and cardiorespiratory control in newborn infants. The original contribution centers on the use of noninvasive methodologies to analyze physiological signals routinely acquired in hospital/home settings. Moreover, the approach to signal analysis incorporates complex system generation and interaction, with a systemic view of the cardiorespiratory physiology. Different modes of interactions were explored, from amplitude to phase modulation. A complex interplay of autonomic regulation, spontaneous contractility of the heart,

peripheral resistance of the vascular tree, effects of circulating hormones and metabolic supply to the heart generates a range of HRV patterns. For this reason, HRV has been for long time considered a powerful tool, to observe non-invasively the interaction between the sympathetic and parasympathetic systems and their capability to respond properly to internal and external challenges. HRV is also influenced by respiration: the neuronal control of breathing and HR are closely linked, functionally as well as anatomically. Thus, breathing and HR are the output of a complex network of controlling mechanisms, which constantly adapt to the ever-changing need of the organism. Many efforts have been spent to describe the systems behind these signals, with time and frequency domain approaches. Nonetheless, the complex origin of these signals makes traditional linear signal processing approaches unsuitable or partially capable of characterizing the systems generating the data. One of the objectives of this thesis was to implement *complex signal processing methods* to characterize newborn HRV and cardiorespiratory activity. For this reason, we selected entropies and phase rectified signal averaging

techniques, given previous positive applications in adults, newborns and fetuses. Our second objective was to characterize and quantify the *mutual influence of cardiovascular and respiratory rhythms*. Many contributions were found in the literature, ranging from cross-spectral analysis to nonlinear methods. Nonetheless, a limitation of all these techniques was that they did not measure the directionality of the relationships. Transfer Entropy (TE) was developed to address precisely this issue. Its focus is on tracking the information flow between two systems. Another approach is that of describing the cardiorespiratory coupling as a dynamic synchronization process, meaning an interaction between two subsystems which can be modeled as two weakly self-sustained chaotic oscillators, quantifying measures like Phase Locking and directionality. These techniques were employed to quantify how SIDS risk factors may alter cardiorespiratory interaction and autonomic activity. We investigated the influence of sleep state, sleep position, prematurity and the combined effect of prenatal smoking and alcohol exposure on baseline physiology and a physiological challenge, i.e. the head up tilt. These conditions were chosen to

cover both intrinsic and extrinsic risk factors, as proposed by the triple risk model, as well as their interaction.

*Effect of sleep states:* Our results indicate that, while Quiet Sleep (QS) generally presents lower global HRV, higher complexity values were observed probably due to increased interactions among physiological systems, as higher TE values indicate. A difference in directionality balance based on sleep state was observed, which could be driven by differences in the average breathing frequency. With respect to the cardiorespiratory synchronization, our findings showed a significantly higher value both in percentage of time spent in phase-coupling and in length of the coupled epochs in QS, both at the newborn and one-month stage. When looking at the parameters behavior as a function of age, the ratio of the dominant synchronization shifted from a majority of 3 beats in 1 breath in newborns, to 4 beats in 1 breath in one-month infants. This is relevant since the peak of SIDS incidence is at 2-4 month of age.

*Effect of position during sleep in premature infants.* Results in the newborn group showed clear differences both in the long and short-term variability parameters, with supine position having higher variability but lower complexity. At 2 months of age, sleep position influenced mostly short-term variability, generally associated with parasympathetic regulation. The fact that at 2 months measures of vagal activity were found to be diminished in prone position suggests a suppressive

effect of this position, changing the sympatho-vagal balance.

*Effect of prematurity:* Results showed increasing mean RR intervals, short-term HRV, HR complexity and linear cardiorespiratory coupling as a function of Gestational Age (GA), indicating a significant increasing cardiorespiratory coupling and autonomic control. GA at birth influenced significantly directionality of phase interaction. In QS all the three GA groups showed the dominant influence of breathing on HR, and this relationship grew with GA. In AS a balanced relationship was present in Late Preterm and it moved toward a dominant relationship from breathing to HR in Full Term. The hypothesis that directionality could depend on respiratory frequency was tested and we found a bimodal influence: concurrently with breathing frequency <0.6 Hz, an increased polarization toward values of directionality from breathing to HR occurred. Nonetheless, the mean breathing frequency did not change significantly in the GA window analyzed (35-40 weeks); thus, the significant change in directionality with GA at birth could not be explained solely by breathing frequency. Our explanation for this phenomenon is that the threshold for the low pass filter effect is still adapting between 35-40 weeks GA. *Effect of prenatal alcohol/smoking exposure on tilt response in newborns:* The expected behavior was displayed only by the unexposed group, with decreases of all the parasympathetic parameters selected after tilt,

while the exposed group instead showed a blunted response. In conclusion, we showed how complex parameters and techniques for bivariate analysis of breathing and HR provide additional information with respect to traditional techniques in the description of how physiological systems dynamically interact to maintain an optimal health status. The proposed novel techniques are advantageous for addressing specific time scales and different modes of interaction for data collected under standard clinical conditions with artifacts and noise. Moreover, these techniques were capable of characterizing systems interactions. This supports our aim to utilize nonlinear advanced parameters to obtain reliable physiological and clinical indices. This approach could lead to a quantitative autonomic profile to assess vulnerability in populations at risk for SIDS. This could grant the possibility of the definition of an "elastic" triple risk model, where the contributions of the various risk factors could be weighted and updated based on infants physical and environmental conditions, hopefully improving predictability and generating novel monitoring solutions.

# PATIENT-SPECIFIC MODELLING OF STENTED CORONARY BIFURCATIONS: A VALIDATED, OCT-BASED RECONSTRUCTION METHODOLOGY FOR CFD ANALYSES

**Susanna Migliori**

**Advisors: Prof. Gabriele Angelo Dubini, Prof. Francesco Migliavacca**

The clinical challenge of percutaneous coronary interventions (PCI) is highly dependent on the recognition of the coronary anatomy that features each individual. The classic imaging modality used for PCI is angiography, but advanced imaging techniques that are regularly performed during PCI, like optical coherence tomography (OCT), may provide detailed knowledge of the pre-intervention vessel anatomy as well as the post-procedural assessment of the specific stent-to-vessel interactions. Computational fluid dynamics (CFD) is an emerging investigation field in the setting of optimization of PCI results. In this study, an OCT-based reconstruction methodology of patient-specific coronary artery models, which include the actual geometry of the implanted stent, was developed for the execution of CFD simulations. The method was developed and validated by means of a rigid phantom that resembled a segment of the left anterior descending coronary artery where a stent was deployed. The methodology comprised the processing of OCT images to retrieve the lumen contour and the stent, then the three-dimensional (3D) reconstruction

was achieved by means of a computer-aided design software. The 3D reconstruction of the stented phantom was assessed with the geometry that was obtained from X-ray computed micro tomography scan, used as ground truth. Results reported twist and distortions that were due to the catheter-based imaging technique, these were reduced with the centreline of one side branch that was chosen as reference for the error angle estimation. The 3D reconstruction was successfully used to perform CFD analyses, demonstrating a great potential for patient-specific investigations. The developed methodology was employed to perform CFD simulations of blood flow across patient-specific coronary arteries with bifurcations after stent deployment. The developed framework was employed to study the post-operative hemodynamics of sixteen patients. Among those a subset of twelve cases underwent OCT acquisitions after 9-months from the intervention. Such images were used to compute the percentage of restenosis area at the scaffolded segment, which was then compared with the distribution of superficial hemodynamics quantities. The superficial distribution of relative

residence time showed similarities with the 2D map of the distance between lumen contours at follow-up and baseline. Such findings were in agreement with the literature, which supported the potential application in cardiovascular intervention. Objectives for future work can be a punctual comparison between fluid dynamics superficial quantities and neointima thickening, enabling for a quantitative study. Chapter 2 introduces the clinical motivation of the fulfilled research work by providing to the reader an overview of the coronary tree anatomy, with atherosclerotic pathology genesis and the main consequences. As the work here reported has an application to coronary bifurcations, a brief insight of the classification accepted by the European Bifurcation Club for the different bifurcation lesions and treatment strategies are described. Additional information about single stent coronary interventions is provided, as these are the resembled scenarios. The second part of the Chapter moves the reader closer to the topic of the dissertation. The information reported seeks to present the challenges of this research field, the novelty of the

developed methodology and the implications to intervention cardiology. This Chapter describes the imaging modalities that are mostly employed during the interventions, focusing on angiography and OCT. The fluid dynamic mathematical formulations implemented for simulating the blood flow are described, alongside the quantities that are commonly considered as descriptors for hemodynamic conditions. Finally, the state-of-the-art in patient-specific modelling is briefly introduced. Chapter 3 presents the developed OCT-based reconstruction methodology and the validation procedure. A typical geometry of a human coronary segment with bifurcations was resembled as a rigid phantom. A metallic stent was deployed in the phantom, then it was imaged with OCT and  $\mu$ CT. This latter provided a reference geometry for validation purposes. A proof-of-concept CFD simulation was performed using the achieved reconstruction. Chapter 4 reports the application of the validated OCT-based methodology to in-vivo medical images that were acquired at the Institute of Cardiology, Catholic University of the Sacred Heart of Rome (Italy). Three patients' angiograms and OCT images were processed to achieve high fidelity 3D geometries of the stented coronary artery segments with bifurcations. Patient-specific CFD simulations were carried out and results were processed for the characterisation of the disrupted hemodynamics after stent implantation. An uncertainty study on CFD boundary conditions

was performed on one case and the results were processed to quantify the effects on the distribution of wall shear stress along the geometry. Moreover, the chapter describes the collaborative work that was performed with partners from the University of Sheffield as part of the European Marie-Sklodowska Curie Innovative Training Network, Virtual Physiological Human-Cardiovascular Simulation and Experimentation (VPH-CaSE). A patient was found with an occlusion at the left main coronary artery bifurcation and underwent PCI procedure with the implantation of one stent. The resultant hemodynamics was analysed with a CFD simulation. Chapter 5 includes the elaboration of pre-operative OCT images for the simulation of highly disrupted blood flow in a coronary artery with spontaneous dissection. This last resulted in the generation of an empty chamber into the vessel wall. The developed reconstruction methodology was employed to achieve the geometry of a right coronary artery segment with bifurcations where an ulcerated plaque was found. Patient-specific CFD simulation revealed heavy flow recirculation in the empty plaque and at the interface with the normal lumen. Chapter 6 presents the application of the validated OCT-based reconstruction method for patients treated with a polymeric bioresorbable scaffold at the Sussex Cardiac Centre, Brighton and Sussex University Hospitals, Brighton, UK. The elaborated data were acquired during a clinical trial whose objectives were the

evaluation of Absorb BVS stent (Abbott Vascular, Abbott Park, IL, USA) at coronary bifurcations. It included the acquisition of intravascular OCT images at baseline, after 9 and 18 months from the intervention. Patient-specific CFD simulations were carried out and the blood flow was characterised in terms of wall shear stress. The OCT images at 9-months follow-up were elaborated to evaluate the neointima thickening which was presented in terms of restenosis area percentages. These last were compared with results from CFD simulations seeking to investigate any relationship between blood flow and vessel remodelling.

# SUPERVISED TISSUE CLASSIFICATION IN OPTICAL IMAGES: TOWARDS NEW APPLICATIONS OF SURGICAL DATA SCIENCE

**Sara Moccia - Advisor: Prof. Elena De Momi**

**Co-Advisor: Leonardo Mattos, Ph.D**

Intra-operative tissue classification plays a fundamental role in different clinical fields. The automatic image-based tissue classification is a valuable solution to provide decision support and context awareness intra-operatively. The surgical data science (SDS) community is focusing more and more on machine learning (ML) to perform tissue classification in the operating room (OR). However, several technical challenges are still present, hampering the clinical translation of the developed methodology in the clinical practice. Indeed, robust and reliable tissue classification is not trivial due to image noise, varying illumination level, different camera pose with respect to the tissues, and intra- and inter-patient tissue variability. There are several aspects that can be tackled to potentially overcome these issues, including hardware design (to allow high-quality optical imaging), identification of images to be processed (to avoid the processing of uninformative images), and estimation of classification confidence (to improve system performance by excluding uncertain results).

On this background, the overall goal of the PhD work was to develop a framework for ML-based SDS algorithms for robust and

reliable tissue classification in intraoperative optical images, as to offer decision support and provide context awareness during interventional-medicine processes. The main contributions (Figure 1) of the PhD thesis are:

- 1) A new method (M1) to automatic learning-based informative frame selection, which exploited a new set of features to retrieve informative frames to be processed by tissue classification algorithms.** M1 exploited intensity, keypoint and image spatial-content features to classify, with multi-class support vector machines (SVM), endoscopic frames as informative, blurred, with saliva or specular reflections, or underexposed. When tested on a balanced set of 720 images from 18 different narrow-band laryngoscopic videos, a classification recall of 91% was achieved for informative frames, significantly overcoming the state of the art approaches (median recall = 57%, maximum recall = 81%).
- 2) A new method (M2) for reliable tissue classification in endoscopic images, which introduced a measure of confidence on**

**classification to retrieve reliable classification results and improve classification performance.** M2 exploited textural information and supervised and semi-supervised approaches coupled with confidence estimation for pathological tissue classification. When tested on 33 narrow-band laryngoscopic videos, which refer to 33 different patients affected by early-stage laryngeal cancer, a median classification recall of 93% was achieved. The method was also tested on the hepatic district, achieving a recall of 82% in classifying 40 RGB images acquired in the OR of healthy and pathological liver donors' grafts. The classification recall was increased to 98% (laryngeal tissue) and to 86% (hepatic tissue) by estimating the confidence of the SVM classification and excluding low-confidence results.

- 3) A new protocol (M3) for endoscopic-image analysis, which exploited multispectral imaging (MI) for data acquisition during endoscopic procedures with the goal of performing robust tissue classification and image tagging.** M3 expanded

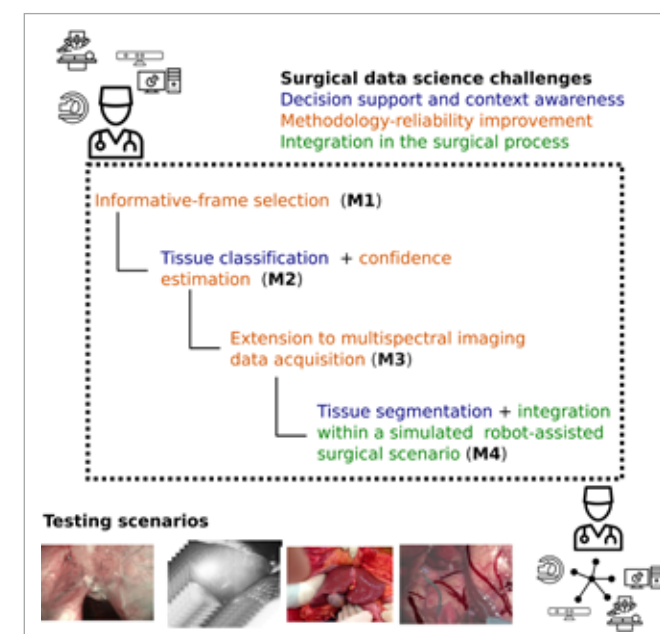
M2 by exploiting for the first time in the literature intra-operative in vivo multispectral-imaging data acquisition and analysis. Multispectral data were acquired during laparoscopic procedures on 7 pigs. Six abdominal organs (abdominal wall, liver, spleen, gallbladder, diaphragm and intestine) were classified with multi-class SVM. The effect of using multispectral data was an increase in accuracy of 11% for the task of organ classification and an increase of 23% for the task of automatic image tagging with respect to standard RGB imaging. When exploiting the confidence measure proposed in M2, the result was a boost in classification accuracy of 38% (RGB) and 20% (MI).

- 4) Integration of the SDS ML-based methodologies within a handheld robotic tool to perform tissue avoidance in simulated robotic-assisted surgical procedures (M4).** With M4, ML-based tissue segmentation was integrated within a simulated robot-assisted surgical scenario. A forbidden-region virtual-fixture control was exploited, as to prevent unwanted robot-tissue interaction. In particular, phantom vascular structures were built and segmented using a deep-learning approach. When including the control, the error on the robotic tip position when it entered the forbidden zone (24% of the time) was small. Indeed,

the median penetration error was 17  $\mu\text{m}$ , which was 2 orders of magnitude smaller than median vessel diameter.

The PhD work lies in the wider research field of SDS, which aims at providing the surgeons with decision support and context awareness during interventional medicine procedures. The methodologies presented in the PhD thesis demonstrated the feasibility of using SDS ML-based algorithms for intra-operative tissue classification in several anatomical districts. The methodological progress made in this work highlights the potential of SDS ML-based algorithms in extracting useful information implicitly contained in intra-operative optical

images, overcoming challenges typical of intra-operative tissue classification to support surgeons during interventional-medicine procedures.



**Fig. 2 - Workflow of the PhD thesis. With the aim to support surgeons during interventional-medicine procedures, machine-learning methodologies were developed to detect and classify anatomical tissues in intraoperative optical images. Several anatomical districts were investigated for testing the proposed methodologies.**

# NOVEL APPROACHES FOR CONTEXT-AWARENESS AND WORKFLOW ANALYSIS IN SURGERY

**Nakawala Hirenkumar Chandrakant**

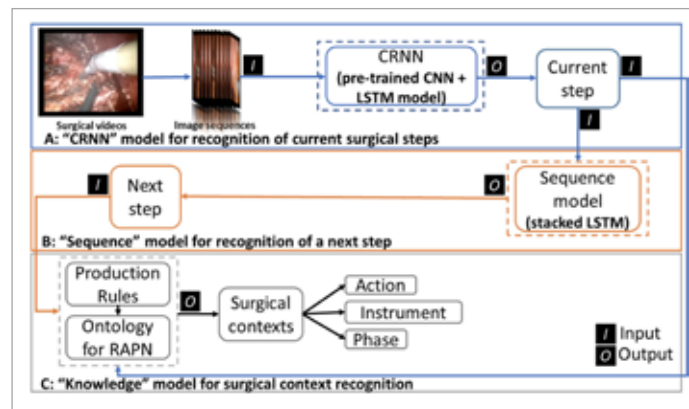
**Advisors: Prof. Elena De Momi and Prof. Giancarlo Ferrigno**

Computer-assisted surgery are increasingly becoming a part of the operating rooms (OR). In future, current OR are expected to include various technologies such as context-awareness and automated surgical workflow analysis with robust sensors, robot-assisted surgical systems, image-guided surgery, virtual and augmented reality systems, and better data analysis and visualization systems. With the inclusion of new technologies e.g. robot-assisted surgeries and change in the conventional surgical approaches, OR are becoming highly involved with numerous sensors, which produce multi-modal information. Despite the discernible advancement of OR technologies, surgeons' decision-making capability has not considerably influenced. Besides, surgeon's cognitive load is increased analyzing such extra surgical representational information, which outpaced human analysis capabilities and leads to preventable medical errors. For the advanced surgeries, surgical simulators are being used for surgical training and replacing the conventional training methods such as lecturing, apprenticeship and video demonstrations. However, current training regime and simulation-based training systems do not automatically

adapt to the training context and surgical workflow. There are several aspects that can be tackled to potentially overcome these issues, including robust software architectures (to allow easy integration of sensor data and surgical information), recognition of contextual information (to guide and train surgeons on surgical workflows and removing unwanted information), and robust surgical workflow analysis (to improve system performance by building and analyzing surgical workflow automatically for context-awareness). Context-aware systems are expected to analyze the intraoperative data and understand the surgical situation automatically, e.g. recognition of surgical steps. Despite being well researched, we

still have not achieved the desired results for context recognition and automatic workflow analysis in surgery. On this background, the overall goal of this thesis is to develop a knowledge-driven context-aware system for reliable recognition and contextual information, aiding assistance to surgical training and providing decision support. The contributions of this PhD work are:

(1) A novel modular knowledge-driven context-aware system framework was developed, which exploits the procedural knowledge and computer vision-based methods to recognise surgical instruments and contextual information. A knowledge-driven context-aware system framework was implemented



**Fig. 1 - "Deep-Onto" network consists of deep learning models for current and next step recognition and knowledge model to identify other surgical contexts**

using a procedural knowledge (in the form of ontology), 3D image processing and semantic queries, which were used to obtain contextual information on the workflow. The framework was able to retrieve contextual information about current surgical activity along with the information on the need or presence of a surgical instrument. To show the applicability, as a paradigmatic scenario, Thoracentesis procedure was chosen. Further to that, based on the framework components, an intelligent training system for Thoracentesis was developed, where formal knowledge about the procedure workflow and entities for object recognition is specified in the form of production rules, which used to recognise surgical context, i.e. surgical instruments and to automatically interpret surgical workflow. Surgical training with the context-aware system showed similar results as mentor-based training. With this framework, knowledge-based context recognition and situation interpretation have been achieved in the pre-operative and intra-operative training scenarios in the laboratory-based setup.

(2) Further to that, in this thesis, a new pipeline was developed which integrates deep learning with knowledge representation techniques, called "Deep-Onto" network as shown in Fig. 1, to recognize surgical workflow. "Deep-Onto" network takes advantage of the formal knowledge and semantic relations encoded inside the ontology to recognize surgical steps and other low-level surgical activity.

The deep learning models were used to recognize the step, which in progress and the consecutive step. After the contextual recognition on the surgical step is achieved, the information was forwarded to ontology to find other low-level surgical entities. We also developed the video annotation dataset on Robot-Assisted Partial Nephrectomy (RAPN), which was used in this work. The system was able to recognize 10 RAPN steps with the prevalence-weighted macro-average (PWMA) recall of 0.83, PWMA precision of 0.74, PWMA F1 score of 0.76, and the accuracy of 74.29% on 9 videos of RAPN. We found that the combined use of deep learning and knowledge representation techniques is a promising approach for the multi-level recognition of RAPN surgical workflow.

(3) This thesis also exploits a new data mining approach using inductive logic programming (ILP) was used to analyze the surgical video annotation data to construct a surgical process model and to analyze the contextual information. We used ILP to automatically develop production rules, in the first order logic, based on the intraoperative video data. These rules were either constructed as to represent the surgical process model or to understand the surgical context by analyzing semantic relations between the surgical entities. Data mining was done on Thoracentesis and RAPN video annotations datasets that were developed during this thesis work. We found that ILP could

learn procedure-specific workflow rules and analyze the contextual information and could a strong technique for context-awareness in surgery.

The overall results presented by this thesis demonstrates that knowledge-driven context aware system, comprising knowledge representation, computer vision, machine learning and logic programming techniques, has potential to be a critical aspect of solving the problem of context-awareness and automated surgical workflow analysis. The progress made in this thesis shows that combining these technologies help overcoming potential barriers to context-awareness in surgery. As a future perspective, this work is a small step in the broader framework of context-aware surgical systems, autonomous and semiautonomous robotic systems to support the surgeon during surgical activities.



# INVESTIGATING VASCULAR INVOLVEMENT IN MULTIPLE SCLEROSIS BY MAGNETIC RESONANCE IMAGING

Laura Pelizzari

Advisors: Giuseppe Baselli, Maria Marcella Laganà, Francesca Baglio

## Background and Aims

Evidence is mounting that vascular factors play an important role in the development of multiple sclerosis (MS). Some studies have reported neck vessel morphological and hemodynamical alterations in MS patients. Furthermore, cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) impairment has been observed. Nevertheless, the exact role that vascular component plays in MS is still not completely understood. Therefore, the aim of this doctoral work was to investigate vascular involvement in MS with magnetic resonance imaging (MRI), from different points of view (i.e. neck vessel morphology, brain perfusion, CVR), in order to provide new insights about this issue.

## Methods

Firstly, a semi-automatic method to measure the cross sectional area (CSA) of major neck vessels on their whole course was introduced for time of flight (TOF) magnetic resonance angiography (MRA) data. A resampling procedure was implemented to normalize CSA measured on all slices, in order to allow group comparisons at homologous levels and on the whole vessel. Then, the intra- and inter-rater

reproducibility of the derived CSA measures was assessed in a group of 36 healthy individuals (HI). In addition, scan-rescan reproducibility was assessed by scanning a group of 9 HI twice, 5 days apart. Once the CSA reproducibility was confirmed, the method was applied in a longitudinal study including 69 MS patients and 22 HI, acquired twice, five years apart. CSA differences were tested at baseline (BL), at follow-up (FU) and over the five years. The association between CSA alterations, MS phenotypes and cardiovascular co-morbidity was tested.

In a separate study, CBF was investigated with arterial spin labeling (ASL) MRI, together with gray matter (GM) atrophy. Specifically, 26 MS patients and

26 HI were recruited to test if hypoperfusion in MS could be related to GM volume loss. Finally, 31 MS patients and 25 HI were scanned with ASL MRI in normocapnic and hypercapnic conditions (Figure 1), and CVR alterations were tested within GM parcels (resting state networks and vascular territories) and white matter (WM). Bivariate correlation between CVR, age and MS clinical parameters was also computed.

## Results

The semi-automatic method CSA evaluation showed excellent inter- and intra-rater repeatability at all cervical levels and on the whole vessel course (ICC>0.90, median DSC>0.9), and good degree of scan-rescan reproducibility for all the considered neck vessels (internal carotid artery - ICA;

vertebral artery - VA and internal jugular veins - IJV) (on the whole vessel: ICC > 0.9,  $p < 0.001$  for CCA-ICAs, ICC > 0.6,  $p < 0.001$  for VAs, and ICC > 0.7,  $p < 0.001$  for IJVs). MS-vs-HI CSA comparison showed no significant CSA differences between the two groups at baseline and at follow-up. Nevertheless, in MS patients, significant CSA decrease was observed for the ICAs (C4:  $p = 0.048$ ; C7:  $p = 0.005$ ; WV:  $p = 0.012$ ), for VAs (C3:  $p = 0.028$ ; C4:  $p = 0.028$ ; C7:  $p = 0.028$ ; WV:  $p = 0.012$ ), and for IJVs (C3:  $p = 0.014$ ; C4:  $p = 0.008$ ; C5:  $p = 0.010$ ; C6:  $p = 0.010$ ; C7:  $p = 0.008$ ; WV:  $p = 0.002$ ). Furthermore, patients with MS without cardiovascular disease showed significantly greater IJV CSA change than MS patients presenting with cardiovascular disease (C3:  $p = 0.018$ ; C4:  $p = 0.018$ ; C5:  $p = 0.010$ ; C6:  $p = 0.015$ ; C7:  $p = 0.018$ ; WV:  $p = 0.003$ ). Significant hypoperfusion was observed in MS patients within the anterior cingulate and paracingulate gyri, supplementary motor cortex, precentral and superior frontal gyrus ( $p_{FWE} \leq 0.05$ ). No GM atrophy was found in these regions. No significant CVR differences were observed in MS group with respect to HI within GM parcels and WM. Significant CVR-age

correlation was found for HI (min RSN  $r = -0.433$ ,  $p = 0.031$ , observed within frontoparietal RSN; min VT  $r = -0.422$ ,  $p = 0.027$ , observed within left posterior cerebral artery VT; WM  $r = -0.488$ ,  $p = 0.025$ ) but not in MS patients. No significant correlation was observed between CVR and lesion load, EDSS, and disease duration.

## Discussion and Conclusions

The first study proposed a semi-automatic method to measure neck vessel CSA along the whole vessel course. The method proved to produce reproducible CSA measures, and the feasibility of CSA longitudinal studies was confirmed. No significant CSA differences between MS and HI were identified at the baseline. However, significant CSA decrease over time of all major neck vessels was observed for MS group, and not for HI. Although Type 2 errors may have occurred preventing showing any CSA difference over time in HC, the significant CSA changes observed in MS patients may be associated to MS-related factors. In addition, a greater CSA reduction was observed for IJVs in patients without CVD with respect to MS patients with CVD, suggesting that the changes in IJV CSA are influenced by MS independently of CVD.

In addition, since the regions where hypoperfusion was found did not coincide with GM atrophy areas, hypoperfusion may not be merely a secondary phenomenon to GM volume reduction. Therefore, brain perfusion might be used as a further marker of tissue damage, in addition to GM volume. Finally, the loss of correlation between CVR and age in the MS group, compared to the significant correlation observed in HI, suggests that other factors related to the disease may influenced CVR in MS.

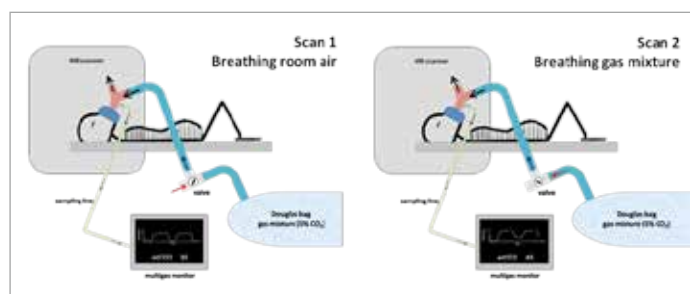


Fig.1 - Experimental setting for ASL acquisition at normocapnia (left panel) and at hypercapnia (right panel).

# THE ROLE OF HYPOXIA-INDUCIBLE TRANSCRIPTION FACTOR HIF-1A IN ACUTE MYELOID LEUKEMIA

Stefano Percio - Advisor: Linda Pattini

With the diffusion and rapid development of high-throughput technologies new data mining strategies, based on biological knowledge and information engineering, have been generated, laying the foundations for the interdisciplinary field of Systems Biology. Specifically, in this study, we applied this holistic approach to investigate the role of the hypoxia-inducible transcription factor 1 alpha (HIF-1A) in acute myeloid leukemia. Generally, leukemias are a composite class of hematological malignancies characterized by a block of the differentiation at different stages of hematopoietic lineage, following by an abnormal proliferation of cancer clones in bone marrow and peripheral blood. Acute myeloid leukemia (AML) is a tumor of the myeloid lineage with a rapid growth, and can be classified based on the maturity of the cell from which leukemia develops, going progressively from the most undifferentiated leukemia (M0) to the most differentiated forms such as M5 or M7. Although only a few effective therapeutic options are available for this disease, and relapse after initial treatment frequently occurs, the identification of novel targets is mandatory. The molecular profiling of different leukemia subtypes could unravel novel

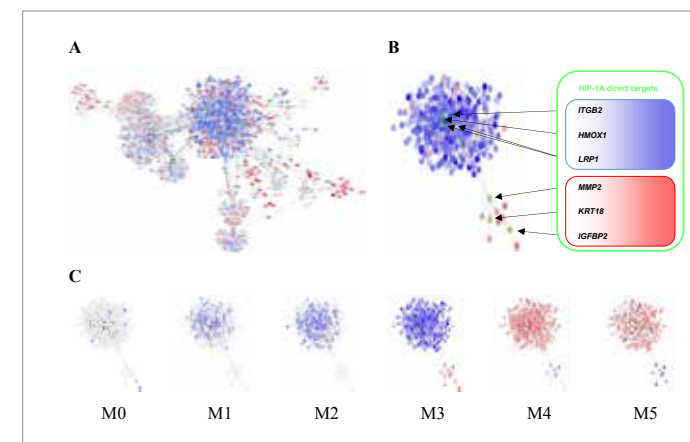
key genes to be exploited as candidates to be tested in clinical trials. In this study, we unearthed a prominent role for the HIF-1A signalling, letting to hypothesize the existence of HIF-dependent/driven leukemia subgroups. This gene is a master regulator of cellular adaptation to a hypoxic microenvironment, but it also prompts a wide range of pathways like invasion, angiogenesis, and tumor development. Previous studies have revealed an interaction between HIF-1A and a chimeric protein, PML-RARA, characterizing a specific leukemic subtype. This subtype is identified as M3, and derived from the promyelocytic stage of the hematopoiesis, therefore called acute promyelocytic leukemia (APL). To this purpose, we started collecting a list of *bona fide* HIF-1A direct targets, and we applied reverse engineering to reconstruct an AML transcriptional network, selecting the genes of the signature as seed. We employed mutual information operator to estimate the pairwise correlation between transcripts since it is able to detect also the non-linear dependencies and it is reparametrization invariant. In a further step, we integrated gene expression analysis to

topological analysis by selecting, only transcripts differentially expressed in APL respect to other AMLs (A). Resulting sub-network showed two distinct communities with a different trend, each one containing three HIF-1A direct target genes (B). Specifically, this pattern evoked typical characters of epithelial to mesenchymal transition (EMT), responsible for initiating metastatic processes through endeavouring the invasive phenotype of cancer cells. In fact, the down-regulated genes are significantly associated to cell adhesion term, while the three up-regulated targets are involved in motility and extracellular matrix degradation. Since genes of the sub-network are mainly down-regulated, we wondered if microRNAs (miRNAs), which have a role in gene silencing, could generate this inhibition. Surprisingly, the *miR-181* family had the widest number of potential targets, and it was recently discovered to promote EMT pathway in ovarian tumorigenesis. Another feature of the sub-network is a clear segregation of the APL samples as emerged by hierarchical cluster. Specifically, a progression in the number of differentially expressed genes seems to take place, with a

peculiar switch between M3 and M4 (C). Moreover, we observed that the same genes showing a peculiar dysregulation pattern across different AML subtypes were also to distinguish malignant from normal promyelocytes, thus ruling out dependence on a myeloid developmental stage. Interestingly, extending the analysis of the signature to the other AMLs, general evidence suggested a heterogeneous HIF-1A involvement in the different subtypes. Employing a class prediction approach, M5 and M0 resulted more discriminated than other AMLs. For M5, which represents the acute monocytic leukemia, we found a prevalence of overexpressed genes, meaning a highly incisive transcriptional

activity of HIF-1A. In addition, due to the presence of genes involved in invasiveness processes and the EMT master regulator *ZEB2*, this subtype exhibited a motility and migratory behaviour. Another EMT master regulator, *ZEB1*, was observed in the subset of genes highly distinctive for M0, the subtype correspondent to the undifferentiated leukemia. Considering the involvement of this gene in a mutually inhibition network motif with some miRNAs well characterized in solid tumors, we were prompted to search for possible candidates also in this context. Due to the overexpression of *ZEB1* in this subtype, of which co-expressed genes were involved not only in EMT, but also in maintenance

of stemness properties, we found three miRNAs coherently down-regulated. All of them are implicated in tumor suppressive pathways in different tumor types. Surprisingly, invasion, cell migration, chemotaxis, and transendothelial migration recurred in the subtypes highly typified by the HIF-signalling. Moreover, we observed that epigenetic modulators such as non-coding RNA and chromatin modifier played a crucial role in EMT-like behaviour promotion and leukemia development. Finally, findings from this study were also confirmed by the HIF-1A inhibition *in vitro*, resulting in impaired bone marrow homing and leukemia progression *in vivo*. In addition, the hypothesis that, in leukemic context, EMT-characters are not a general phenomenon of morphological transformation within the myeloid lineage, but rather segregate with specific leukemia subtypes targeting master regulators triggering EMT, and could represent a promising strategy to further increase the efficacy of AML treatment. Overall, our data suggest that, in the era of precision medicine, selected patients could benefit from combination approaches with HIF or EMT inhibitors to reduce leukemia dissemination.



**Fig. 1 -HIF-1A-dependent sub-network is specifically dysregulated in APL.** A) Transcriptional network of gene interactions correspondent to the statistically significant pairwise dependencies of AML bone marrow expression data. Each circle represents a transcript. Down-regulated transcripts are in blue while up-regulated transcripts are in red. B) The sub-network was obtained by extracting from the overall AML network only transcripts that are differentially expressed (FDR  $q$ -value  $< 0.05$ ) in the comparison between APL (M3) and other AML subtypes. Down-regulated transcripts are in blue while up-regulated transcripts are in red. Six HIF-1A direct target genes (highlighted in green) are present: MMP2, KRT18, and IGFBP2 in the up-regulated community, and ITGB2, HMOX1, and LRP1 in the vast community mostly down-regulated. C) The APL-specific sub-network observed across different AML subtypes (from M0 to M5). Down-regulated transcripts are in blue, up-regulated transcripts are in red, non-differentially expressed transcripts are empty circles.

# SINGLE CELL FLUID DYNAMICS FOR THE STUDY OF THE LIVER MICROCIRCULATION: COMPUTATIONAL MODELLING AND *IN VITRO* VALIDATION

Monica Piergiovanni - Advisor: prof. Gabriele Dubini

The development of computational tools to support biological research is now a central topic in the field of biomedical engineering. This is particularly crucial in those applications where experimental methods are not used and/or do not provide a complete view of the process. This is the case when studying the immune response to some pathologies of the liver, where the biochemical mechanisms studied *in vivo* on animal models need to be supported also by a fluid dynamic investigation at the level of microcirculation. The present dissertation is aimed at the development of computational fluid dynamics (CFD) models to be applied to liver capillaries - called sinusoids, organised in the liver functional unit, the lobule - and at the study of cell motion inside them.

The first model presented, reproduced a portion of the sinusoidal network, by means of an adequate reconstruction protocol applied on images captured *in vivo* on a mouse liver. To set the pressure in the eleven outlet sinusoids, a lumped parameter model was built, representing the sinusoid portion until the central vein. Resistances were calculated with a Poiseuille law with measures from the images stack. Velocities -  $90 \div 360$

$\mu\text{m/s}$  - and pressure - 187Pa- were in good agreement with literature data. The model was also used to evaluate the effect of an adhered leucocyte on the fluid dynamics of one of the branches. Wall shear stress on the sinusoidal/cellular wall are greatly influenced, reaching a disturbed condition with peaks of 5 Pa of the shear stress for the highest occlusion rate tested (Figure 1). This approach was able to accurately reproduce the local fluid dynamics at several stages of the leucocyte adhesion.

A Volume of Fluid (VoF) model, specific for the study of two-phase flows, was then built to account for the flowing of a cell in micro channels. With this model, a single cell - leucocyte or Red Blood Cell (RBC) - was

represented as a fluid droplet, with specific physical properties. This model was used to investigate the effect of the cell presence on the fluid dynamics of the whole microchannel, by varying several parameters, specific of the liver microcirculation. Mainly, the ratio between the cell and the plasma viscosity played a crucial role on the deformation of the cell. Less viscous cells (Red Blood Cell) tended to deform when flowing in capillaries twice as big as them (Figure 2), while more viscous cells (leucocyte) rotated and shifted towards the capillary wall, until they reached an equilibrium position. The VoF model provided all fluid dynamic parameters that, combined in a post processing algorithm, gave the state of stress on an

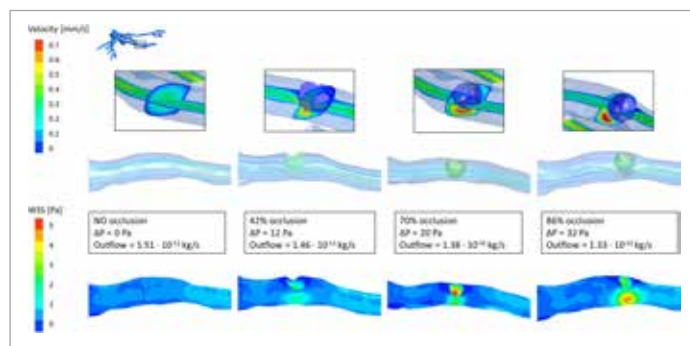


Fig. 1 - 3D method: effect of leukocyte adhesion on WSS and streamlines, contributing with different occlusion percentage (from left to right: no occlusion, 42%, 70% and 86%). Estimated pressure drops created by the adhered cell and outflow from the occluded branch are also reported.

infinitesimal element in a flowing Newtonian fluid. From this, it was possible to estimate the forces acting on the cell membrane due to the simple flowing in a capillary network ( $111 \div 203$  pN for the RBC and  $797 \div 849$  nN for the leucocyte), an information that cannot be inferred by any existing experimental methods.

This VoF model was validated with an experimental campaign on cell deformed under a shear flow in microfluidic devices, designed fabricated for this specific application. By capturing images of cells with a high speed camera, the deformation of HL60 and Jurkat cells was estimated at varying flow rates -  $25 \div 125$   $\mu\text{l/min}$  - and compared to computational simulations (Figure 3). At increasing flow rate, the cell is progressively deformed by the fluid from a circular to an elliptical shape up to a ratio = 1.35 while maintaining a constant area (about  $70 \div 80 \mu\text{m}^2$ ). Even if the cell populations showed a great variability in terms of deformability due the biological difference among single cells, a statistically significant difference could be found at varying flow rates. The computational model was able to represent the deformation of a single cell in an extensional flow, with minor underestimation due to the 2D approximation.

Finally, the VoF model was integrated with the sinusoidal network model, by reconstructing the computational domain from the *in vivo* images and modelling a single cell flowing in one of the branches. This preliminary simulation showed

the feasibility of using the VoF model for application in the liver microfluidics. By means of this model it was also possible to estimate the shear stresses and forces acting both on the cell and on the endothelium, with values ranging from 11.8 to 19.5 pN.

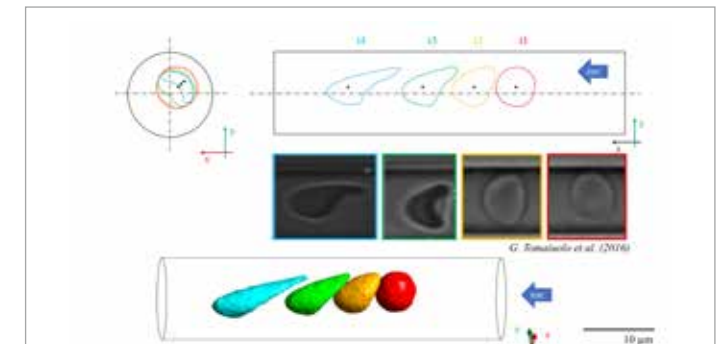


Fig. 2 - Results of VoF model for RBC model pRe 6:36 104;Ca 3 102; 1:5;D{H 0:5q. Deformation and equilibrium position at four time step -  $z = 31, 41, 62, 83 \mu\text{m}$  - and comparison with literature (Tomaiuolo et al., 2016)

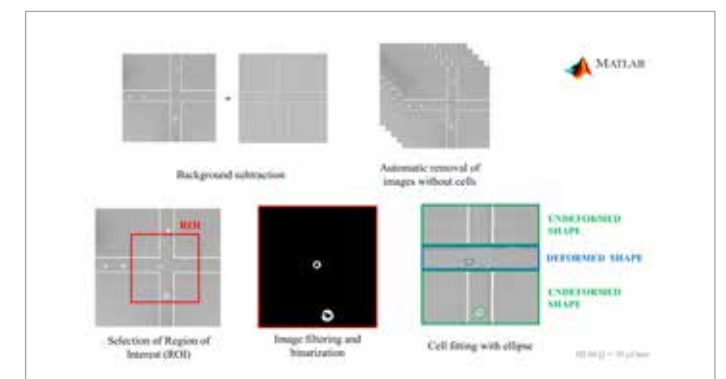


Fig. 3 - Post processing flow (images refer to HL60 at a flow rate of 30  $\mu\text{l/min}$ ). Top panel: automatic background subtraction and empty images removal. Bottom panel: definition of a ROI, image binarization and ellipse fitting.



# MODELING OF MICROVASCULATURE IN UREMIC PATIENTS

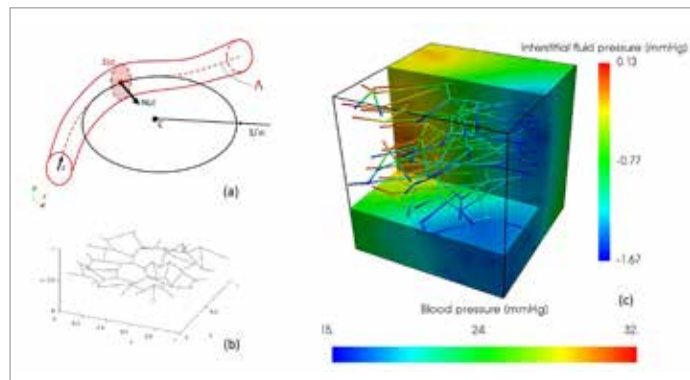
Luca Possenti

Advisors: Maria Laura Costantino, Paolo Zunino

Microvascular alterations have recently been reported in patients affected by chronic kidney disease treated by hemodialysis therapy. How these microvascular alterations are related to the pathology is still an open and complex question. Such alterations are likely to be related to the non-physiological flow rates of fluids and solutes removed from blood in the artificial filter. The artificial treatment increases the instantaneous mass- and fluid-flow rates which have to be washed out from the interstitium into the blood compartment, thus over-stressing the microvascular wall membrane. This membrane alteration affects the fluid balance, the distribution of solutes, and the delivery of nutrients to the tissues and may contribute to an abnormal vascular development and morphology. In addition, the presence of uremic toxins (currently more than one hundred have been identified) induces further alteration on the microvascular membrane wall. However, very few studies have addressed how these toxins affect the microvasculature. To address this complex scenario, a wide modeling approach was designed. It is composed of three different models, which can share information and results to better describe the complex phenomena

involved: (i) lumped parameter model of the arterial circulation; (ii) multiscale 3D-1D model of the microvasculature; (iii) *in vitro* model of the microvasculature. The lumped parameter model was based on previous works describing the arterial circulation including peripheral vascular districts and vascular regulations. A single peripheral district was analyzed to detail its description with particular reference to the fluid balance. Tests have been conducted by considering some uremic parameters alterations and highlighting the need for a comprehensive modeling approach. The second computational approach consists in a multiscale model of the microvasculature,

accounting for its geometry and solved by finite element method. It was developed under the supervision of Prof. Paolo Zunino, MOX laboratory, Department of Mathematics, Politecnico di Milano. It exploits a framework of partial differential equations on domains with different dimensions (3D for the interstitium and 1D for the vasculature). Based on previous works on advanced mathematical methods and their development, the proposed model accounts for a non-linear contribution of the lymphatic system, the rheological effect of red blood cells and their heterogeneous distribution along the vascular network. The model was tested on a number of different cases

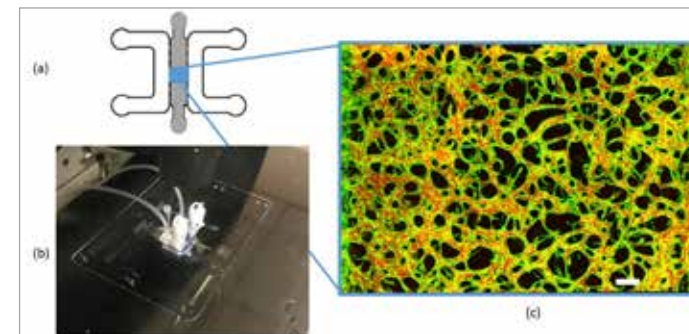


**Fig. 1 - 3D-1D model of the microvasculature. (a) Reduction to 1D for a generic curved vessel. (b) Example of an artificial generated computational network. (c) One of the results obtained in this work. A portion of the interstitial domain has not been displayed in order to show the microvascular network.**

before being applied to address uremic microvasculature, leveraging on available data from literature. Tests have assessed the contributions of the different relevant factors and how test-driven results actually match with *in vivo* literature-driven data. Moreover, a sensitivity analysis was conducted to appreciate the effects induced by the alterations related to the pathology. Thanks to this analysis, the central role of the capillary wall hydraulic conductivity was highlighted, particularly motivating the *in vitro* analysis, which is aimed at describing its alterations. The *in vitro* model was developed at the Mechanobiology Lab, Massachusetts Institute of Technology (Cambridge, MA, USA) under the supervision of

Prof. Roger Kamm and thanks to the collaboration with the Cell and Tissue Engineering Lab at IRCCS Galeazzi Orthopaedic Institute (Milan, Italy) - Dr. Matteo Moretti. The Roberto Rocca Doctoral Fellowships (Spring 2018) supported the collaboration with MIT. This *in vitro* model consists in the generation of microvasculature on a microfluidic chip, achieving a 3D microvascular network which can be perfused and pressurized. It was proposed to analyze alterations of the capillary membrane induced by the presence of uremic toxins. To this aim, a trans-mural pressure was applied to study microvascular wall properties. In addition, the experimental setup was computationally analyzed, by using

the 3D-1D model, to investigate the actual rise of the trans-mural pressure between the vasculature and the gel surrounding it. Finally the models' improvements and their interactions have been discussed, along with their limitations. This work has paved the way for a modeling support to the current research activities on microvascular alterations in uremic patients.



**Fig. 2 - The *in vitro* model: microvasculature on a chip. (a) Geometry of the chip used in this work. (b) Picture of the experimental setup on the confocal microscope. (c) *In vitro* microvasculature on a chip perfused by dextran solution. Green: GFP positive endothelial cells. Red: Dextran solution. White bar: 200  $\mu\text{m}$ .**



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

Roberta Visone

Advisors: Prof. Marco Rasponi and Prof. Alberto Redaelli

Heart diseases still represent a leading cause of death worldwide due to the limited capacity of mature cardiomyocytes to proliferate and regenerate necrotic myocardium. The ability to model human cardiac physiology *in vitro* would represent a breakthrough in the investigation of key biological aspects involved in heart development and disease progression, possibly paving the way to new strategies for drug discovery and providing new hints in the field of regenerative medicine for the development of new cardiac therapies.

Bioengineering approaches are strongly needed in the relatively new field of human induced pluripotent stem cells (iPSC). In particular, new technological tools are expected to provide significant environmental conditioning for iPSC manipulation and to enhance currently limited- *in vitro* maturation of cardiomyocytes derived from them (iPSC-CMs). The present PhD Thesis aimed to develop novel technological solutions to design advanced cell culture platforms, both at the macro (i.e., Bioreactors) and micro scale (i.e. Organs-on-Chip). By tailoring native cardiac-specific cues, the ultimate goal was the generation and testing of physiologically relevant and functional three-dimensional (3D)

*in vitro* cardiac cellular models. A biomimetic macroscale bioreactor will be presented, featuring an innovative geometry of the cell culture chamber, designed to provide cardiac patches with a bidirectional interstitial perfusion combined with a precisely controlled electrical stimulation. The peculiar design of the chamber allowed for the first time to directly evaluate individual cardiac patch during culture, assessing its maturation, either by optical inspection or through direct tests of contraction (Figure 1). Of note, these measurements could be carried out without interrupting the culture operations. The bioreactor was validated with culture neonatal rat cardiomyocytes seeded on collagen scaffolds; the effects of a biomimetic electrical stimulation coupled with

bidirectional perfusion on patch maturation were assessed. Subsequently, microfluidic bioreactors will be presented, each designed to address specific limitations of current Organs-on-Chip devices. An innovative biomimetic microfluidic platform was developed, able to integrate for the first time biochemical, electrical and mechanical conditioning of 3D cardiac microtissues. An electrical system was embedded in a microfluidic platform designed to provide controlled mechanical stimulation (i.e., uniaxial strain) to 3D cardiac microtissues. Stainless steel electrodes were used both to provide a tightly controlled electric field, to stimulate microtissues in culture, and to perform pacing tests, assessing their beating synchronization level.

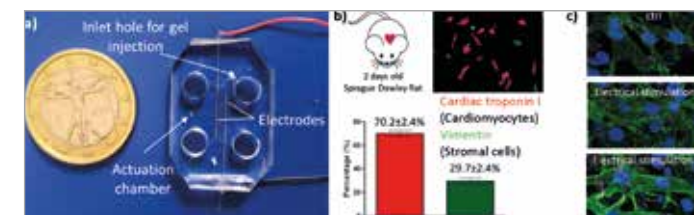


**Fig. 1 - a) The bioreactor allows the release of a single culture chamber b) to perform the live imaging of labeled cells of the cardiac construct by means of a fluorescence microscope.**

This platform was used to study the effect of different electrical stimulation patterns, alone or combined to mechanical stimuli, on neonatal rat cardiomyocyte microtissue functionality and maturation. Furthermore, a new technology allowing for the first time the generation of multiple 3D cardiac microtissues within microfluidic platforms in an easy and uniform fashion, was developed. Microtissues were generated from iPSC-CM and exploited in developmental biology studies and drug screening assays in a mid- to high-throughput fashion. Microfluidic platforms were thus developed and validated as alternative tools to standard 2D cell culture systems, allowing to better reproduce physiological cell responses. Finally, a cardiac-specific microvasculature model will

be presented. The model was developed to better represent *in vitro* the interplay occurring *in vivo* between different cell types (i.e., fibroblasts and endothelial cells). By exploiting human endothelial cells and cardiac fibroblasts, functional and perfusable vasculature networks were developed and characterized. The collected preliminary data on vessel growth and maturation will serve as a basis to further studies on myocardial vasculogenesis. In the present PhD thesis both technical and biological novelties were achieved. Technological advancements comprised: *i)* the improvement of the biomimetic stimulation strategies in both macro and micro bioreactors (i.e. coupling different physical cues of native cardiac milieu) and *ii)* a new technique enabling for efficient generation of multiple tissues for high-throughput drug

screening. These platforms were pivotal in the establishment of functional cardiac microtissues, and eventually exploited to get new biological insights related to cellular responses when subjected to combinations of physiologically relevant stimuli.



**Fig. 2 - a) Developed microfluidic platform for the electro-mechanical stimulation of 3D microtissues. b) Initial cell population characterized from immunofluorescence images to be composed of cardiomyocytes (70.3%±2.4%) and stromal cells (29.7%±2.4%). c) Immunofluorescent staining (cardiac Troponin I in green; Connexin-43 in purple) of the electrically stimulated cardiac microtissues (low voltage (LV) and high voltage (HV)). Scale 10µm.**