MECHANICAL ENGINEERING / PHYSICS / PRESERVATION OF THE ARCHITECTURAL HERITAGE / STRUCTURAL, SEISMIC AND GEOTECHNICAL ENGINEERING / URBAN PLANNING, DESIGN AND POLICY / AEROSPACE ENGINEERING / ARCHITECTURE, BUILT ENVIRONMENT AND CONSTRUCTION ENGINEERING / ARCHITECTURAL URBAN INTERIOR DESIGN / BIOENGINEERING /DATA ANALYTICS AND DECISION SCIENCES / 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 (μBS Lab, DEIB), Computational Biomechanics Lab (DEIB), Biocompatibility and Cell culture Lab (BioCell, CMIC), Bioreactors Laboratory (CMIC). The Istituto di Elettronica, Ingegneria dell'Informazione e delle Telecomunicazioni (IEIIT) of the Consiglio Nazionale delle Ricerche (CNR), which is located at DEIB, represents another possible option.

Stage periods in distinguished research institutes in Italy and abroad are an essential feature of the PhD candidate training. The candidates are encouraged to carry out part of their research activities in contact with other research groups, preferably abroad through periods of at least three months spent in laboratories where the candidate can acquire further skills to develop his/her research work and thesis. Collaborations that may involve the PhD students are presently active with several national and international research and academic Institutions. Very often, the involvement of companies and clinical partners facilitates the technological transfer of applied research into industry and clinical applications.

The educational offer includes *ad hoc* advanced courses specifically designed for the PhD in Bioengineering. The offer includes also the school of the National Bioengineering Group, which is held yearly for one week in Bressanone (Bz). Every year, the School is focused on different topics. As examples, the themes of the last few years have been: Neuro-informatics (2011), Biomedical devices from research to market (2012), Regenerative medicine (2013), From functional recovery to artificial organs (2014), Experimental models for development methods for 3R (2015), Bioengineering for Active ageing (2016), E-Health and digital medicine (2017), Biomedical Images (2018), Technologies and tools in surgery and therapy (2019), AI-enabled health care (2020).
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.

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.

COMPOSITION OF THE PHD BOARD	
Aliverti Andrea (Coordinator)	DEIB
Bianchi Anna Maria	DEIB
Boschetti Federica	CMIC
Candiani Gabriele	CMIC
Cerveri Pietro	DEIB
Cimolin Veronica	DEIB
Corino Valentina	DEIB
Costantino Maria Laura	CMIC
Dellacà Raffaele	DEIB
De Momi Elena	DEIB
Draghi Lorenza	CMIC
Dubini Gabriele	CMIC
Farè Silvia	CMIC
Ferrante Simona	DEIB
Fiore Gianfranco Beniamino	DEIB
Gastaldi Dario	CMIC
Guazzoni Chiara	DEIB
Mantero Sara	CMIC
Pattini Linda	DEIB
Pozzi Giuseppe	DEIB
Ravazzani Paolo	CNR
Redaelli Alberto	DEIB
Rodriguez Matas Jose Felix	CMIC
Signorini Maria Gabriella	DEIB
Soncini Monica	DEIB
Villa Tomaso Maria Tobia	CMIC

VIRTUAL REALITY-BASED MULTIDOMAIN INTERVENTIONS FOR OLDER ADULTS WITH MILD COGNITIVE IMPAIRMENT

Sara Arlati - Supervisors: Giancarlo Ferrigno, Marco Sacco

Mild Cognitive Impairment (MCI) is neurological condition characterized by the impairment of one or more cognitive domains; nonetheless, it still does not compromise the autonomy of the individual. MCI often represents a prodromal stage of dementia, but it can also revert back to a normal cognitive status. Because of this, MCI population represents the optimal target to administer interventions aimed at improving cognitive outcomes, and halting the progression of symptoms of dementia.

To treat MCI, research is focusing on multi-domain non-pharmacological interventions targeting dementia risk factors. In such a context, Virtual Reality (VR) has emerged as a promising tool, because it allows for training in ecological scenarios that can be adapted to each user's needs. VR is also engaging and able to increase the motivation to train. To do this, it exploits *Sense of Presence* (SoP), i.e., the feeling of "being there'' in virtual environment. SoP is dependent on objective and subjective factors, among which there is the degree of immersion provided by the visualization device. More immersion – e.g., in the case of head mounted displays (HMDs) – generally results in higher SoP; however, it may also cause *cyber-sickness*, a set of symptoms including nausea, blurred vision, and disorientation.

Due to these reasons, this thesis addresses the topics of feasibility and acceptance of VR-based interventions to improve cognitive outcomes in older adults with MCI. The work followed a pathway going from the study of non-immersive applications, to most immersive ones.

The first studies addressed the feasibility of a VR-based program coupling Physical Exercise (PE) and Cognitive Training (CT). A system based on a touch-screen TV and a cycle-ergometer implemented 3 scenarios: (1) a park in which the users travelled while performing controlled aerobic PE; (2) an urban scenario with congested cross-roads; (3) a supermarket in which to search for grocery items among distractors (Figure 1).

Fig. 1 - One participant performing cognitive training with the supermarket scenario.

The pilot study foresaw 15-20 minutes of PE and 25 of CT (cross-roads + supermarket) for 6 weeks, 3 times a week. Ten volunteers aged >65 with mild dementia were randomized to an intervention (IG) and a control group (no treatment, CG). Post-intervention, IG showed a tendency toward improvement in cognitive outcomes. A significant improvement in physiological outcomes (reactive oxygen species concentration: +8% in IG, -4% in CG; creatinine: -4% in IG, 0% in CG) indicated that the program was effective in reducing biomarkers of Alzheimer's Disease. The program was well accepted by all participants, who felt engaged thanks to VR. They also reported an improvement of their Quality of Life (QoL), resulting from reduced anxiety in their daily life. The second study was a randomized controlled trial with three groups (n=20 each) receiving either CT, PE or CT+PE for 12 weeks, and controls (n=20) who received no treatment. Significant improvements in cognitive outcomes were found for the groups who received CT: Short Story Delayed Recall was improved in CT+PE post-training ($F(3,76)=2.82, p=0.045$), and in CT+PE and CT at follow-up. Phonological fluency ($F(6,152)=2.254, p=0.041$) and depression ($H(3)=1.826, p=0.05$) improved over time for CT group too. Results showed also high acceptance: 95% of participants would have continued with the training, and many reported improved QoL.

The second set of activities explored the increase of immersion (and of SoP) while cycling. Since the navigational component in immersive VR may cause cyber-sickness, a preliminary trial was conducted enrolling healthy young adults. The previously-mentioned park and road-crossing scenarios were modified to run on a HMD. Results showed that the occurrence of cyber-sickness could be masked by higher SoP, since participants, despite side-effects, preferred the experience with the HMD. Nonetheless, cyber-sickness was too high to consider this setup safe for older adults. Thus, the HMD was replaced with a Cave Automatic Virtual Environment (CAVE). A cognitive task was also added, implementing a Dual Task paradigm. Five older adults who tried the experience of cycling in the CAVE for 15 minutes reported no side-effects, and enjoyed the experience. Therefore, the CAVE-based training could represent a valuable solution to administer immersive VR-based PE to older adults.

After having sought the best way to administer PE to older adults, the acceptance of an immersive HMD-based supermarket experience was also explored. As for the park, a preliminary study was performed, but, in this case, cyber-sickness was very limited. Whether interacting with VR causes the use of more cognitive resources, and if it can distract the users from the main (cognitive) task, was also assessed. Ten healthy young adults, and 3 older adults were thus asked to reach for grocery items in real world (RW), real world while holding the HTC Vive controller (RWC), and virtual reality (VR). Analyzing motion data, it emerged that movement times were significantly longer in VR; however, interjoint coordination and almost all joints' ranges of motion were not affected by the HMD, indicating that movement patterns were preserved, and thus that possibly no additional workload was required. Given this, a study to assess immersive supermarket acceptance was finally conducted on a sample of 57 older adults with cognitive complaints (Figure 2). The experience, lasting 15 minutes, was well accepted (TAM3 score: >5.33 out of 7), and was capable of eliciting high SoP (ITC-SOPI spatial presence: 3.51 ± 0.50, engagement 3.85 ± 0.68, naturalness 3.85 ± 0.82); only minor side-effects were recorded (SSQ score: 3.74/0-16.83).

Fig. 2 - A participant immersed in the Virtual Supermarket

This work assessed the feasibility of VR-based interventions providing PE and CT, and the acceptance of immersive VR interventions aimed at improve cognitive outcomes in older adults with MCI. VR has confirmed a promising means to administer CT and PE to older adults, and its flexibility should be further exploited to design customized intervention. Immersive VR was well accepted too, but a good design is essential to avoid cyber-sickness, especially for older adults. This work had some limitations that may reduce the generalizability of results; nonetheless, the promising outcomes suggested that the application of (immersive) VR to cognitive interventions is worthy of further investigations.

MRI–BASED RADIOMIC ANALYSIS OF RARE TUMORS: OPTIMIZATION OF A WORKFLOW FOR RETROSPECTIVE AND MULTICENTRIC STUDIES

Marco Bologna

The main purpose of this thesis was the optimization of a workflow for the radiomic analysis of Magnetic Resonance Images (MRI) acquired with uncontrolled image acquisition protocols. The secondary aim was the application of the optimized workflow to build prognostic models for Overall Survival (OS) for Head and Neck Cancer (HNC) and Soft Tissue Sarcoma (STS), in order to show the feasibility of using radiomics in multicentric and/or multiprotocol datasets.

The first part of the work focused on a series of stability analyses performed using a virtual phantom (BrainWeb). The aim of these studies was two-fold: 1) to evaluate the effect of image preprocessing on the stability to imaging-related variability; 2) to select the features that are stable to such variations, in order to use them for the following analysis. Intensity standardization, image denoising, voxel size resampling and bias field correction were considered as potentially useful preprocessing steps. Intraclass Correlation Coefficient (ICC) was used to quantify features stability, and features with ICC>0.75 were considered stable. All the preprocessing steps (Gaussian filtering, N4ITK Bias field correction, B-spline spatial resampling and intensity standardization) had positive effects in increasing the stability of radiomic features. When including all the previous preprocessing step, 550 features, based on both T1-weighted (T1w) and T2-weighted (T2w) MRI were identified as stable, out of a total of 1072 (536 per image type). Stability to uncertainties of the region of interest (ROI) was also investigated. Two sources of variability were considered: multiple segmentation and geometrical transformations of the ROI. Both tests were performed on real images of STS and HNC, considering T1w, T2w and apparent diffusion coefficient maps (ADC). In each test, features with ICC>0.75 were considered stable. In total, 701 and 1057 features out of 1608 were stable for HNC and STS respectively. After properly combining these stable features sets with the results previously obtained on the BrainWeb dataset, the number of stable features was reduced to 410 and 617. These two sets of features were used for successive studies.

The postprocessing of the features was also optimized. In particular, features normalization and feature selection/dimensionality reduction were optimized in order to maximize the performance of a Cox proportional hazard regression model. Four different features normalization algorithms and 2 different features selection pipelines were tested. Harrell's C-index was used to quantify the models' performance. It was found that the combination of Z-score normalization and a series of different features selection (pairwise correlation and cross-validated Multivariate-Cox) lead to the best performance in a retrospective multicentric HNC dataset (C-index 0.67).

After the optimization based on the results of the previous analyses, the radiomic workflow was used to identify signatures that were prognostic of OS in HNC and STS. In HNC, a five-features radiomic signature had a good prognostic value in both cross-validation (C-index 0.67) and independent validation (C-index 0.63) and in both cases the radiomic features improved the prognosis when added to the clinical ones (from 0.67 to 0.69 and from 0.69 to 0.72 for the cross-validation and independent validation respectively). Similar results were found after cross-validation of a radiomic model in STS (C-index 0.74, 0.74 and 0.78 for the radiomic, clinical and combined model).

The results show that with the right processing, radiomic analysis from non-standardized images is possible and provides a consistent improvement in the prognostic performance of survival model for OS.

MULTI–PARAMETRIC IMAGING IN PARTICLE THERAPY: INTEGRATING MACRO & MICROSCALE MODELS

Giulia Buizza - Advisors: Guido Baroni, Chiara Paganelli

Charged particle therapy (CPT) is a type of radiation therapy that makes use of charged particles, such as protons (PT) and carbon-ions (CIRT), to improve the treatment therapeutic window. Despite its clinical advantages, historical and economic factors limited its usage in the past. With the current surge in CPT centers being built, it is even more relevant to improve tumor response and reduce toxicities to organs at risk (OARs), by better stratifying patients and better predicting response to treatment. With the studies described in this thesis, we show how this can be achieved by exploiting imaging, which can non-invasively characterize the underlying tissue, and modelling strategies, relying on either statistical or machine learning approaches.

1) **Radiomics for survival analyses in CIRT** – Radiomic features from multi-parametric imaging and dose maps, along with clinical variables, were explored as prognostic factors for local control (LC) in skull-base chordoma (SBC) patients undergoing CIRT. Radiomic MRI, CT and dose features, were fed to Cox and survival support vector machine models, using single or combined modalities. The output was evaluated through the concordance index on validation and test sets and exploited to stratify patients in high- and low-risk groups, for which survival curves were built and compared. Multi-parametric imaging and treatment-related features, combined with clinical information, showed promising results as prognostic factors for LC in SBC treated with CIRT. Future studies are needed for validation on larger datasets.

2) **Diffusion-(DWI) and perfusion-weighted imaging for patient stratification in PT** – Relying on pre-treatment data from 26 patients affected by meningioma and enrolled for PT, correlations between dynamic susceptibility contrast (DSC) and intravoxel incoherent motion (IVIM) perfusion parameters, and their usefulness as imaging biomarkers were investigated. As IVIM and DSC are optimized to be sensitive to different tissue characteristics, parameters from the two sequences appeared to be weakly correlated. In this patient cohort, IVIM parameters were shown to be promising biomarkers for meningioma grading.

3) **DWI for treatment monitoring in PT** – A multi-parametric MR protocol was prospectively defined to monitor the impact of fractionated PT on healthy and tumor tissues. Data was collected from 20 patients affected by meningioma before, during (10th, 20th, 30th fractions), and 3 and 6 months after treatment. IVIM parameters were extracted for both tumor and white matter (WM) regions, with the latter being sub-divided according to the dose received. For tumors, only diffusion parameters significantly changed during the treatment. In terms of WM, most of significant changes were found at late time-points and in low dose regions, which showed opposite trends (increase) with respect to intermediate and high dose regions (decrease). With this study, early effects of radiation on both tumor and OARs were preliminarily evaluated towards a more comprehensive evaluation of CPT effects, that could guide treatment adaptation and dose boosting protocols.

4) **Tumor control probability (TCP) models using DWI in CIRT** – In a retrospective cohort of 20 patients treated with CIRT for whom apparent diffusion coefficient (ADC) maps were available, cellularity was computed from ADC and radiosensitivity parameters were estimated from an observed dose-LC relation. These patient-specific parameters were fed into a TCP model (TCP_{ADC}) that was compared to a standard one (TCP_{LIT}). The personalized TCP_{ADC} suggested more conservative estimates with respect to TCP_{LIT}, which agrees with studies showing that SBCs highly benefit from dose escalation protocols. The prognostic power of the two TCP models, assessed through a ROC analysis, was shown to be equivalent, but results must be validated on a larger patient cohort.

5) **Tumor characterization and monitoring in PT through DWI** – To better understand how the a-specific ADC relates to cancer tissue microstructure, a computational method was developed and employed for grading and response monitoring tasks. DWIs from before and three months after PT were collected for patients affected by meningioma, for whom the risk of an adverse outcome was inferred by their clinical history. DWI signals were also simulated from realistic synthetic cells' packings. Patient and simulation data was coupled through a LASSO-regularized optimization that allowed estimating markers of tumor microstructure, namely diffusion coefficient (D), volume fraction (vf) and radius (R). The apparent cellularity (ρ_{app}) was estimated from vf and R for clinical interpretation (Fig.1). Significant differences in ADC, D, vf, R and ρ_{app} values were found between meningothelial and atypical subtypes, and between WHO I and II/III grades. F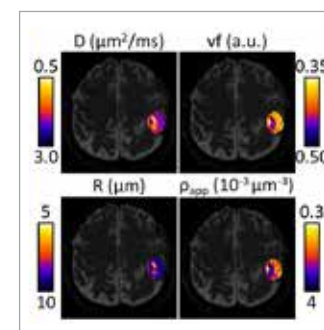rom ROC curves, the estimated microstructural parameters showed higher specificity than ADC. The skewness of ρ_{app} achieved the best separation of high- and low-risk patients. Even if further validation is needed, a set of potential imaging microstructural markers for grading and response monitoring in PT was proposed, implying important advantages for patient-specific PT workflows.

6) **Range verification in PT** – Due to specific CPT characteristics, electromagnetic beam properties could be exploited for real-time range verification. Proton beams were simulated using the Geant4-DNA toolkit to evaluate the impact of distinct physical interactions on the current density associated with the beam. Low energy electrons were found to mostly impact the current density in the transversal plane, whereas they introduced a noticeable but limited effect along the major beam axis.

7) **Technical validity of DWI for biomarker studies** – Recommendations from the Quantitative Imaging Biomarkers Alliance (QIBA) were followed to acquire DWI from an ice-water phantom. Requirements were met on the clinical scanner from which the data used in this thesis was acquired, supporting its technical robustness.

Fig. 1 - Estimated diffusion (D) and microstructural (R, vf, ρ_{app}) parameters for a patient affected by meningioma.

CHARACTERIZATION OF NF-κB DYNAMICS IN A MODEL OF TUMOR/MICROENVIRONMENT INTERACTION USING A COMBINATION OF SINGLE-CELL LIVE IMAGING AND MICROFLUIDICS: INSIGHTS FROM MULTIPLE MYELOMA.

Federica Colombo - Supervisor: Marco Rasponi

Co-supervisor: Alessandra Agresti

NF-κB is a family of transcription factors involved in cells survival, cytokines production and immune response. In physiological condition, NF-κB resides inactive in the cytoplasm of cells; however, extracellular inflammatory signals can activate NF-κB that translocates into the nucleus and promotes the transcription of several genes, including its own inhibitors, which act as negative feedback loops to avoid NF-κB sustained activation. Deregulation of NF-κB is associated to initiation and progression of the second most common haematological malignancy in Italy, Multiple Myeloma (MM). In this PhD project we aimed at characterizing, for the first time, NF-κB dynamics in living MM and bone marrow stromal cells to better comprehend MM biology. A bioengineering approach with cutting-edge techniques, as quantitative single-cell live imaging and microfluidics, was developed for this purpose.

NF-κB dynamics were investigated by live imaging in p65 (NF-κB subunit) YFP knocked-in cells obtained by CRISPR/Cas9: p65 YFP MM.1S (myeloma) and p65 YFP HS-5 (bone marrow stroma). Both cells were subjected to static (autocrine/paracrine signaling) and continuous (autonomous signaling) inflammatory stimulation provided by TNF-α. We demonstrated that, upon static stimulation, stromal cells were able

to resolve p65 nuclear translocation after 8 hours of live imaging, while MM.1S cells maintained a sustained p65 activation; in continuous inflammatory stimulation provided by commercial microfluidics, both cells exhibited a comparable and more sustained p65 activation, meaning that extrinsic factors influenced p65 modulation differently in stromal and myeloma cells in static setting. What is supposed to happen in bone marrow of MM patients is an hyperactivation of NF-κB in the tumor microenvironment, due to released factors from both MM and stromal cells. Starting with 2D macroscopic MM-stroma co-cultures, we were not able to distinguish any influence on p65 activation when the cells were together. A possible explanation could be related to the macroscopic environment provided by the wells, where autocrine/paracrine signals

from the co-cultures can be highly diluted. This is the reason why, to investigate how stromal cells-mediated paracrine signaling acts on NF-κB in myeloma cells, a 2-layer custom-made microfluidic device was designed and validated. The first layer (culture layer) is composed of two symmetrical channels for 3D cultures of MM.1S and HS-5 in fibrin gels, to minimize the volumes and recreate the 3D ECM-like material around bone marrow environment. The two separated 3D cultures can be connected using Doormat valves controlled by the top layer (pneumatic layer) (Figure 1). The device was validated in terms of chamber independence maintenance (when valves are closed) and minimum pressure for opening the valves with evaluation of diffusion time. From a biological point of view, unstimulated cells plated in a 3D fibrin environment
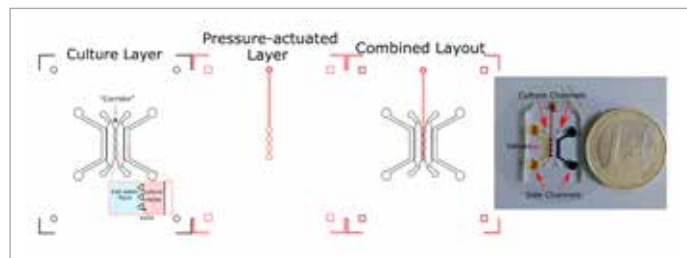
were less stressed than in 2D cultures and did not exhibit NF-κB activation caused by the new 3D environment. Additionally, NF-κB dynamics reflected the trend observed in 2D culture: in unstimulated setting, myeloma cells showed higher basal level than stromal cells; TNF-α stimulation activated both cell types, while IL-1β elicited p65 translocation in HS-5 only. The most striking result obtained was the heterogeneous activation of p65 in myeloma cells once the paracrine factors from stromal inflammatory environment reached the MM compartment, upon the opening of the valves (Figure 2). Although only a fraction of MM.1S was activated by stromal released factors, this result was not achievable in 2D macroscopic co-culture, highlighting

the importance of new user-friendly *in vitro* solutions to better mimic the tumor microenvironment.

In conclusion, in this PhD thesis, we presented a complete toolkit of innovative approaches to investigate NF-κB dynamics in Multiple Myeloma and bone marrow stromal cells. Overall, this characterization and optimized set-up could lead to a deeper knowledge of MM biology with the hope to find, in the future, new molecular players for pharmacological intervention.
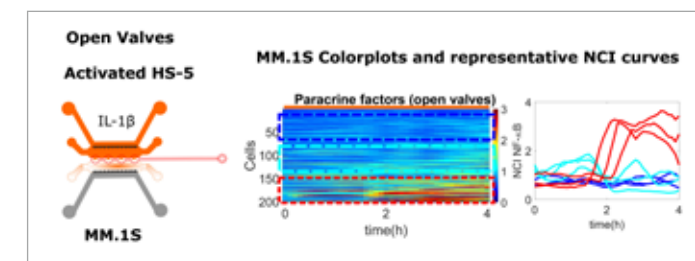

Fig. 1 - On the left, the culture layer, composed by two symmetric culture channels+side channels. In the red rectangle a zoom of the culture channel and pillars: cell-laden fibrin is injected in the inner channel, while side channels are used for culture media. The pressure actuated compartment composed of a series of 4 round normally-closed valves is represented in red. On the right, the combined layout with the culture layer as bottom layer and the valves as top layer and the actual device realized in PDMS.

Fig. 2 - On the left, the experimental scheme: the orange color shades in the device represent the diffusion of secreted molecules from HS-5 that have been stimulated for 2 hours with IL-1β before valves actuation (IL-1β activates NF-κB in stromal cells only). In the middle, NCI (nuclear/cytosolic p65 intensity) colorplot of p65 dynamics in MM.1S followed for 4 hours after valves actuation. Each row is a cell and the color refers to level of p65 activation (blue-low, red-high). MM.1S upon diffusion of factors from stroma showed high heterogeneity: blue and cyan rectangles indicate not or very mildly activated cells, respectively. There was a fraction of MM.1S which was clearly activated (red rectangle). On the right, NCI curves of 3 cells for each subpopulation indicated by the rectangles.

OPTIMIZED CURVILINEAR PATH PLANNING AND POSE ESTIMATION FOR A PROGRAMMABLE BEVEL-TIP NEEDLE IN KEYHOLE NEUROSURGERY

Alberto Favaro - Supervisors: Elena De Momi, Ferdinando Rodriguez y Baena

Over the last decades, a great interest has been shown towards the development of flexible steerable needles in minimally invasive surgery. These needles feature complex kinematics that hinders the possibility to plan the insertion trajectories unless with the aid of an automatic path planner. Solutions proposed in the literature for automatic steerable needle path planning in 3D focus either on a fast computation to allow the interactive re-planning or on path optimality at the expense of high computational time.

The needle motion plan can be executed by a robotically-assisted insertion platform. During the needle insertion, the control system needs to know the needle position and orientation in order to address for possible needle torsion that has been experimentally proven to affect percutaneous needles undermining the insertion accuracy. Because of the thin needle diameter, current tracking systems can not sense the torsion of the needle about its insertion axis.

On this background, the overall goal of this PhD thesis is to describe a preoperative curvilinear path planner for steerable needles and to design a solution for estimating the needle tip position and orientation (i.e., the full pose) during the insertion. In particular, the contributions of this PhD work are:

1. *A pre-operative curvilinear path planner for steerable needles able to solve the planning problem computing a kinematically-feasible path. The planner optimizes the solution according to the criteria of minimum path length and maximum obstacle clearance keeping the computational time consistent with standard pre-operative planning algorithms.*

To contextualize the planning problem with respect to the state of the art, a literature review on path planning for steerable needles is reported, with a focus on the widely used sampling-based methods. A pre-operative curvilinear path planner is then presented. Through a bespoke evolutionary optimization, the planner can maximize the obstacle avoidance while minimizing the path length. In addition, by defining the subspace of reachability of the needle and confining the path search within this region, the algorithm achieves a computational time consistent with standard pre-operative planners. The solution was validated through multiple simulated needle insertions in a neurosurgical scenario.

2. *An on-line pose estimation solution for a multi-segment steerable needle*
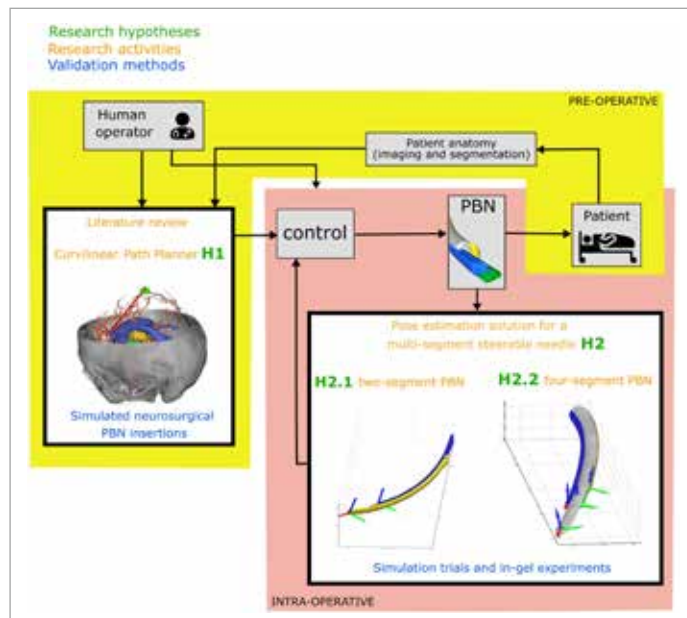

Fig. 1 - Graphical abstract of the PhD thesis reporting the research hypotheses that guided the PhD work, the performed activities and the solutions employed to validate the proposed methods.

using position measurement from sensors mounted on the needle tip.

A solution for the accurate estimation of the needle pose is presented, based on the kinematic model of the needle and position tracking data. The position of the needle segment tips are retrieved by electromagnetic sensors and used by a kinematic-based prediction method to correct the needle state estimation and infer the angle of needle torsion. The method was tested on a two-segment steerable needle in simulation and in phantom-brain gelatine. A reliable and robust estimation was demonstrated with position and orientation errors consistent with the state of the art. The solution was later extended to a four-segment needle. Ingel validation shown the feasibility of the method although, in the latter case, a long time of convergence was evidenced for the torsion angle.

The Programmable Bevel-tip Needle (PBN) is a multi-segment steerable needle under development within the EU EDEN2020 project. It is composed of four axially-interlocked slender sections, robotically actuated to develop specific tip configurations that allow the needle to steer in the space. In this PhD dissertation, the PBN is considered as a case study for the presented methods.

NOVEL APPROACHES FOR THE ANALYSIS OF ATRIAL ACTIVITY IN ECG RECORDINGS OF PATIENTS WITH ATRIAL FIBRILLATION

Rita Laureanti - Advisor: Luca Mainardi

Atrial Fibrillation (AF) is a common heart arrhythmia characterized by a pathological, uncoordinated atrial depolarization. AF causes structural and electrical remodeling in the atria, thus favoring the persistence and worsening of the arrhythmia over time. Indeed, AF usually starts from a paroxysmal form, in which the normal sinus rhythm (SR) is restored within seven days from onset. Paroxysmal AF eventually progresses to persistent (abnormal heart rhythm continues for more than a week, including episodes requiring treatment for termination) and permanent AF (no further attempt to restore SR). The presence of AF increases the risk of suffering from adverse events such as myocardial infarction, heart failure and stroke, as well as the risk of death.

A variety of ECG parameters have been presented in literature to identify patients prone to develop AF, considering both atrial and ventricular activity. Less information is present about the possibility to use such parameters to discriminate between the different stages of AF. This knowledge could be useful to select the best treatment for a new patient and perform long-term monitoring of the pathology. In addition, while several studies consistently demonstrated the deteriorating effect of irregular ventricular contractions during AF on hemodynamics, less is known about the prognostic value of the atrial activity and its variability.

The characteristics of the atrial signal during AF have been used to assess the probability to restore sinus rhythm (spontaneously AF termination or probability of successful cardioversion/ablation). However, the ability of these parameters to predict adverse events has been assessed only in a cohort of patients with congestive heart failure. Even less is known about P-wave variability parameters and the risk of adverse outcome.

Aim of the thesis is i) to assess whether ECG-related parameters present significant differences among AF types, and ii) to evaluate the association between atrial ECG parameters and the risk of suffering from adverse events related to AF, such as stroke, heart failure, myocardial infarction and

cardiovascular death.

Being the AF marked by an irregular atrial activity, the thesis assessed not only the atrial parameter themselves but also their variability over 5 minutes, suggesting novel features for the atrial analysis of AF. For the recordings with SR as main rhythm, the spatio-temporal beat-to-beat P-wave variability was computed. For ECGs with AF, after cancellation of the ventricular activity, the fibrillatory signal was analyzed in both the frequency and time domain. In particular, in the frequency domain the atrial fibrillatory rate (AFR), organization index (OI) and exponential decay (ExpDecay) were computed on the spectral profile; in the time domain the sample entropy, the standard deviation and the amplitude of the fibrillatory waves
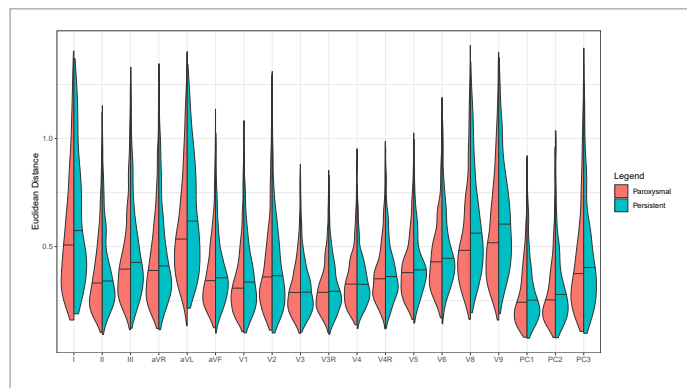

Fig. 1 - For each lead and principal component, the distributions of the beat-to-beat P-wave variability measured as Euclidean distance are depicted for the paroxysmal group (red) and persistent group (green). The median value is depicted by a black line. The difference was significant for leads I, III, aVR, aVL, V1, V6, V8, V9 and PC2.

were computed on windows of 10 seconds, together with their median absolute deviation (MAD) across all windows. The analysis was performed on the Swiss-AF population, a cohort with 2415 enrolled patients with paroxysmal, persistent or permanent AF, availability of 5-minute 16-lead ECGs and information about occurrence of adverse events in follow-up.

The results indicate that atrial parameters show significant differences between AF types. In particular, during SR the beat-to-beat P-wave variability was significantly higher in the persistent group than in the paroxysmal one (e.g. 0.33±0.22 vs 0.30±0.19, p=0.003 in V1, see Figure 1). For recordings in AF, the AFR and OI showed significant differences between the groups in both V1 and lead II: the AFR was lower and

the OI higher for paroxysmal AF in comparison to both persistent and permanent AF. Furthermore, in lead II the f-wave amplitude was significantly higher in paroxysmal AF than in persistent/permanent AF, whereas the standard deviation of the atrial signal was significantly higher in the paroxysmal group, but only when compared to permanent AF.

In the second part of the thesis, significant associations were found between occurrence of adverse events in follow-up and atrial parameters. For SR recordings, it was found that a longer PQ was correlated to higher risk of heart failure, whereas AF patients with shorter P-wave had a higher risk of suffering from stroke. Moreover, higher beat-to-beat P-wave variability was associated with higher risk of cardiovascular death and heart failure. On the contrary, myocardial infarction was associated to lower beat-to-beat variability. During AF, it was found that a lower ExpDecay was associated to higher risk of cardiovascular death (see Figure 2) and lower AFR and higher OI were associated to higher risk of heart failure and cardiovascular death. Regarding the temporal analysis in V1, a higher value of the MAD of the sample entropy and a lower value of the MAD of the f-wave amplitude were associated with higher risk of stroke. In summary, this thesis aims to present a comprehensive study on

the ECG metrics able to describe the abnormal atrial activation that characterizes AF in order to evaluate their effectiveness in tailoring treatment and predicting prognosis in the clinical context. The focus on atrial parameters and their variability allows the direct assessment of the substrate that initiates and maintains AF, playing a fundamental role in the AF analysis.
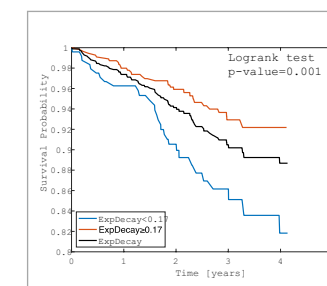

Fig. 2 - Survival probability estimated by the Kaplan-Meier method according to the value of the ExpDecay. The logrank test was applied between the 2 curves obtained splitting the population according to the threshold value of 0.17.

CONTROL STRATEGIES FOR A BACK-SUPPORT EXOSKELETON TO ASSIST WORKERS IN MANUAL MATERIAL HANDLING

Maria Lazzaroni - Supervisors: Elena De Momi, Jesús Ortiz

Occupational back-support exoskeletons are being developed and introduced in the workplace in order to reduce back-related musculoskeletal disorders associated with the execution of manual material handling activities. Existing evidence tends to confirm the benefits of back-support exoskeletons for preventing low back pain associated with the execution of manual material handling tasks that involve trunk flexion/extension or static bending postures. Compared to passive devices, active exoskeletons are considered more versatile, because of the possibility to modulate the assistance during the operation. Effective modulation of the assistance is made by means of appropriate control strategies. Considering the industrial application, the assistance provided by an exoskeleton should adapt according to the different tasks performed by workers. In particular, each specific task implies different movements and thus different assistance requirements. An active back-support exoskeleton has the possibility to implement multiple control strategies in the same device and to interchangeably use them to assist the current task the user is performing. The present work contributes to enhancing the versatility of active back-support exoskeletons by proposing solutions for assisting different manual material handling tasks executed in the

workplace. The aim is to improve assistance effectiveness and users' acceptance for exploiting the support of an active back-support exoskeleton in a wider range of applications. Considering the industrial workplace, the main factors to be considered when selecting a control strategy are its practical functionality and usability (regarding user's residual mobility, physical comfort, whole device encumbrance, and ease of use) and its effectiveness in reducing musculoskeletal disorders risk factors. In this context, the core contributions of this doctoral research addressed two manual material handling tasks, namely lifting and lowering tasks and pulling task. Starting from the results obtained with the biomechanical analysis, a control strategy for assisting lifting and lowering tasks was implemented based on the user's trunk angular acceleration. The control strategy presented, by taking into account the dynamics of the user's movement, is able to adapt the assistance to the different phases of the tasks. In particular, this control strategy improves the pattern of the assistive torque by reducing the hindrance perceived by the user when flexing the torso in the lowering phase and increasing the support in timing with the user's need (i.e., beginning of lifting). The strategy effectiveness was experimentally evaluated relative to the condition without the exoskeleton as well as against existing strategies

for comparison. Using the exoskeleton during lifting and lowering tasks reduced the peak compression force on the L5-S1 disc by up to 16%, with all the control strategies. Substantial differences between the control strategies in the reductions of compression force, lumbar moment and back muscle activation were not observed. However, the speed reduction for the dynamic control strategy appears to be lower compared to the other strategies, although no statistical significance was found. This result encourages further investigation as it seems to support our initial hypothesis that the new control strategy provides more appropriate support to the tasks, improving the timing of the assistance in relation to the typical dynamics of the movement, with positive improvement in intuitiveness and comfort in use, and limiting the exoskeleton's negative impact on productivity (i.e., the hindrance to fast movement is reduced).

In the second place, this thesis proposes the first control strategy to assist the execution of pulling task. A preliminary control strategy was designed, based on the activation of the user's forearm muscles. The assistive torque modulated with the forearm muscle activity is expected to adapt to the user's need of assistance and in particular to the mass of the pulled object, as the activity of

forearm muscles is considered to be an indication of grip strength. An experimental evaluation was performed to assess the effects of the pulling strategy on assisting the execution of the task. Objective measurements, in terms of users' back muscle activation reduction, show the promising benefit provided by the exoskeleton assistance. By reducing the activation of these muscles during the execution of the task, their contribution to lumbar compression is expected to decrease.

Fig. 1 - The XoTrunk is an active back-support exoskeleton designed to reduce lumbar loading on users performing manual material handling activities. It is a rigid exoskeleton that weighs approximately 6 kg and is powered by an onboard battery. Two electric actuators are aligned with the hip joints axes of flexion-extension and generate the assistive torques in the sagittal plane to support hip and back extension. The assistive torques are transmitted to the wearer as assistive forces applied onto his/her torso and thighs, through the body attachments.

A FRAMEWORK FOR THE EVALUATION AND IMPROVEMENT OF HUMAN ERGONOMICS IN HUMAN-ROBOT COLLABORATION

Marta Lorenzini - Supervisors: Elena De Momi, Arash Ajoudani

Work-related musculoskeletal disorders (WMSDs) are impairments of the human body structures that are provoked or worsened primarily by work and by the effects of the immediate environment in which work is conducted. WMSDs are the leading cause of disability in four of the six World Health Organization regions, with substantial economic costs and a severe impact on the quality of life. The careful monitoring of workers' exposure to the factors which may contribute to their development is of crucial importance in industrial environments, aiming to lay the foundation of risk prevention and reduction programs. Nevertheless, in the brand-new industrial scenario, featured by frequently varying workflows and unstructured work stations, the traditional view of occupational ergonomics is rather weak and barely applicable. In fact, the most widely used tools are still "pen-and-paper" observational approaches, which need to be carried out in an off-line stage. On the other hand, numerous techniques have been proposed by researchers to estimate humans' physical load, relying on direct measurements collected on the human body through sensors devices. But complex laboratory-based approaches are hardly personalisable and impractical for industrial settings. Accordingly, the scientific objective of this thesis is to fill in this gap, by introducing a

novel framework for the evaluation and improvement of human ergonomics, which implements online, personalisable, and reconfigurable strategies to account for workers' ergonomic demands. The proposed framework entails three main components: the observation layer, the warning layer, and the action layer. Within the observation layer, data about the humans' motion and the interaction forces they exchange with the environment are measured with fit-for-industry sensor devices, and a subject-specific model of the human body is identified. Their integration enables to define and estimate a human ergonomics monitoring system. The latter is a comprehensive set of indexes to assess humans' physical exposure, accounting online for multiple ergonomic risk factors to the development of WMSDs. Both kinematic and dynamic aspects are addressed, taking into account the whole-body human. To validate the proposed monitoring system, an experimental analysis is conducted on twelve subjects considering three different tasks, which represent typical jobs in manufacturing industries and, additionally, are associated with different potential risk factors. As a result, the ergonomic indexes that better explain the physical load required in each analysed activity are established, confirmed by the outcome of a surface electromyography (sEMG)

analysis. Within the warning layer, the levels of the ergonomic risk associated with the estimated indexes are determined. Then, by taking advantage of intuitive and practical feedback interfaces (i.e. visual and vibrotactile), this information is conveyed to the workers to improve their risk-awareness. Both the proposed solutions prove their potential in assisting humans in their occupational activities through corrective feedback interfaces. Within the action layer, an optimisation procedure is adopted to estimate a more ergonomic human body configuration by minimising a selected ergonomic index according to certain constraints. Subsequently, a worker can be facilitated to achieve such an optimal condition by following the guidance of a collaborative robot, thus mitigating the effect of the associated risk factor (see Figure 1).
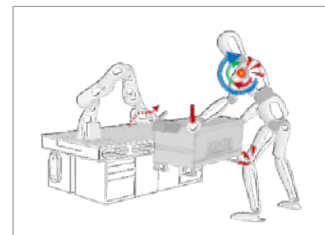

Fig. 1 - The human-robot collaboration framework to guide a worker toward a more ergonomic body configuration and mitigate the physical effort.

The experimental investigations conducted to evaluate the performance of this human-robot collaboration (HRC) framework provide evidence of its capability to reduce the effort on human joints, due to the robot reactive behaviour. Such findings are supported by the results of a sEMG analysis. The proposed HRC strategy shows promising capabilities to reduce humans' exposure to the factors that may determine WMSDs, ensuring workers' well being while enforcing productivity. Therewith, its key strength in the applicability to realistic industrial environments is exhibited.

MICROFLUIDICS AND BIOFABRICATION TECHNOLOGIES TO STUDY AND MODULATE OSTEOARTHRITIS-RELATED INFLAMMATION IN 3D MICROENVIRONMENTS

Carlotta Mondadori

Supervisors: Alberto Redaelli, Marco Rasponi, Silvia Lopa, Matteo Moretti

Osteoarthritis (OA) is the most common type of arthritis and affects millions of people worldwide. OA induces the progressive degeneration of the articular cartilage and of the surrounding tissues leading to the loss joint functionality and to a high disability. Nowadays, the current OA therapies are based on symptomatic drugs that do not counteract the disease and, at later disease stages, surgical joint replacement represents the only possible option. Recently, the inflammation of the synovial membrane, called synovitis, has been shown to play a crucial role in the onset and progression of OA pathology, with OA synovial membrane showing a consistent infiltration of macrophages. In this context, the search for new therapies targeting inflammation and focusing on the macrophagic cell component may represent a promising approach for the treatment of this inflammatory disease.

In this context, the use of advanced 3D models mimicking the interactions between joint tissues and the complex mechanisms of OA pathology represents a promising tool in view of finding novel OA therapeutic approaches. To this aim, in Chapter 3, we designed and fabricated a microfluidic platform that includes the compartments representing all the main tissues involved in synovitis: the vascularized synovial membrane, the articular cartilage, which is the main target of inflammatory processes, and the synovial fluid that contains pro-inflammatory molecules secreted by tissue-specific cells in the joint. In the design phase, our attention was specifically focused on the dimension of the channel mimicking the synovial venule, which was sized to mimic the shear stress reached in the synovial venule in vivo. The developed microfluidic platform was used in Chapter 4 to recapitulate the mechanism of monocyte extravasation that leads to the abnormal accumulation of macrophages in the synovial membrane. The idea was to reproduce monocyte extravasation in view of targeting this process to develop novel strategies for OA treatment. Synovial fibroblasts and articular chondrocytes were isolated from human OA tissues to model, respectively, the synovial membrane and the articular cartilage. These tissue compartments were separated by a channel for OA synovial fluid injection. The endothelialized channel comprised in the synovial compartment was stimulated by fluid flow and by a pro-inflammatory factor to mimic the in vivo situation. The developed osteoarthritic joint-on-a-chip model was then used to reproduce monocyte extravasation in response to chemoattractant factors. We demonstrated that monocytes specifically extravasated only in the presence of chemokines and that extravasation was enhanced when the endothelium was pre-activated by both fluid flow and inflammatory stimulus. Following the hypothesis that OA synovial fluid contains inflammatory molecules that can promote the recruitment of monocytes, we also provided for the first time a direct evidence that OA synovial fluid induces monocyte extravasation.

Besides therapies targeting monocyte extravasation, another approach to regulate inflammation is represented by the modulation of macrophage phenotype once macrophages infiltrate the synovial membrane. Based on environmental stimuli, macrophages can polarize toward an M1 or an M2 phenotype, thus exerting pro-or anti-inflammatory properties, respectively. The idea was to exploit the anti-inflammatory potential of M2 macrophages to modulate inflammation. Besides biochemical factors, also biophysical cues have been shown to play a role in influencing macrophage behavior. Specifically, scaffolds characterised by specific 3D geometric cues have recently emerged as a promising approach to control macrophage phenotype. To this aim, in Chapter 5, we exploited the Melt Electrowriting (MEW) technique to generate Polycaprolactone (PCL) scaffolds with different 3D architectures to study their influence on macrophage response in terms of cell morphology, surface marker expression and secretion of pro- and anti-inflammatory proteins. Different architectures based on different pore geometries were generated: square, triangle and rhombus. Macrophages showed a different morphology when in contact with different scaffold architectures in correspondence of angles: macrophages formed "bridges" in the square and triangle scaffolds, while in the rhombus this did not occur. On the contrary, rhombus enhanced the elongation of cells along fibers. Among all architectures, the rhombus was the only one that enhanced the secretion of all the analyzed anti-inflammatory proteins (IL1RA, IL10, IL13, CCL22, CCL24), with a significant difference for IL10 compared to PCL films.

To summarize, in the present PhD thesis, we presented two different approaches that can be pursued for the development of novel therapies counteracting OA-related inflammation. In one case, therapies aiming at blocking monocyte recruitment to the synovial membrane can be developed to prevent the negative consequences caused by the abnormal infiltration of monocytes. In the other case, therapies aiming at exploiting the anti-inflammatory properties of macrophages can be developed to promote a tissue healing-friendly environment.

PHYSIOLOGY BASED MACHINE LEARNING AND DATA ANALYTICS FOR PERINATAL MONITORING – A NOVEL FRAMEWORK FOR A COMPREHENSIVE MATERNAL, FETAL, AND NEONATAL PROFILING

Nicolò Pini - Advisor: Maria G. Signorini

The perinatal period plays a crucial role in shaping early stages of life and has profound repercussions that attain the potential to extend beyond early childhood. This intuitive and common-sense evidence is supported by recent research which extensively documented several associations between perinatal health and chronic diseases of adulthood. In this Ph.D. thesis we designed an innovative methodological framework towards a comprehensive, longitudinal, and rigorous characterization of the diverse phases of the perinatal period as well as we modeled the interrelationship among the diverse involved subjects. We proposed a novel and rigorous contextualization of the perinatal period aimed to describe this crucial developmental period as a whole by means of multisource information fusion and advanced monitoring. We utilized an ensemble of computational approaches aimed to provide interpretable models encompassed machine learning, artificial intelligence, and advanced signal processing techniques towards prediction of pathological states in the fetal period, data imputation, non-parametric clustering of maternal substance exposure, network physiology analysis and risk assessment in newborns. Furthermore, the novelty of this Ph.D. thesis lies in its analytic framework integrating heterogenous data

sources. Specifically, quantitative data such as fetal, neonatal, and maternal physiological signals and extracted physiology based parameters are contextualized with qualitative data such us maternal lifestyle reports, and precise and timely quantification of exposure. A roadmap of the machine learning and artificial intelligence model ensemble is shown in Figure 1. We started this investigation by analyzing a pathological fetal condition accounting for a considerable portion of the perinatal causes of increase morbidity and mortality, namely intrauterine growth restriction (IUGR). Specifically, we proposed to develop a set of models for the in-utero detection of the pathology in contrast with an at birth assessment. The innovative approach encompassed the design of a more comprehensive framework for the promptly identification of potential risk of fetal growth restriction utilizing data integration and multivariate machine learning approaches. This was achieved by complementing the preexisting knowledge of physiology based heart rate features with maternal information. The methodological aspect of contextualization described in this Ph.D. thesis allowed to create models able to account changes in fetal autonomic regulation throughout pregnancy yet producing results independent of the time of fetal assessment. This technical solution

promoted the generalization of the framework and its insensitivity to potential differences in time of assessment between healthy and pathological fetuses.

The subsequent investigated aspect of pregnancy encompassed an unsupervised and data-driven framework for the imputation and clustering of maternal exposure data. The limitation of imprecise and missing data on maternal substance use/abuse in pregnancy represents a crucial limitation for the quantification of their effects on perinatal states of increased risk. Among the modifiable maternal conditions contributing to the insurgence of fetal pathological conditions, exposure to alcohol and tobacco plays a substantial role. The two foremost perinatal pathological conditions induced by in-utero exposure are the insurgence of IUGR condition and late prematurity. The integration of poorly estimated maternal exposure features in any given model is expected to underestimate and undercount their contribution to the quantification of potential states of risk. To effectively contextualize fetal and neonatal wellbeing with information on maternal habits in the perinatal period, we proposed the utilization of advanced data-driven computational methodologies. Specifically, we addressed the design of a technique for the imputation of missing data on alcohol and tobacco consumption. The

imputation of longitudinal data, and more in general missing information is a complex and multiparametric problem. The innovative approach proposed in this Ph.D. thesis consists of the utilization of a KNN-based imputation. The absence of constrain to fit the available data within a given parametric distribution allowed to decrease variance and potential bias on the derived estimates. A family of methodologies expected to benefit from a more precise characterization of maternal exposure to substances of abuse is clustering. Furthermore, from a methodological perspective, clustering can be interpreted as methodology for data dimensionality reduction, thus reducing computational burden of subsequent machine learning and

artificial intelligence approaches. Feature contextualization plays a central role in the investigation presented in this Ph.D. thesis. As previously discussed, pregnancy, delivery, and early childhood may only be interpreted in the each other's context. Late prematurity is often classified of subclinical relevance given the gestational age at birth of babies in this group is at most two weeks smaller than the clinical cutoff of 37 weeks. Nonetheless, their risk of exposure to mortality and morbidities is significantly higher compared to terms babies. We proposed to build a signal processing framework able to perform risk stratification making use of advanced signal processing methodologies. The differences among late, early, and full term
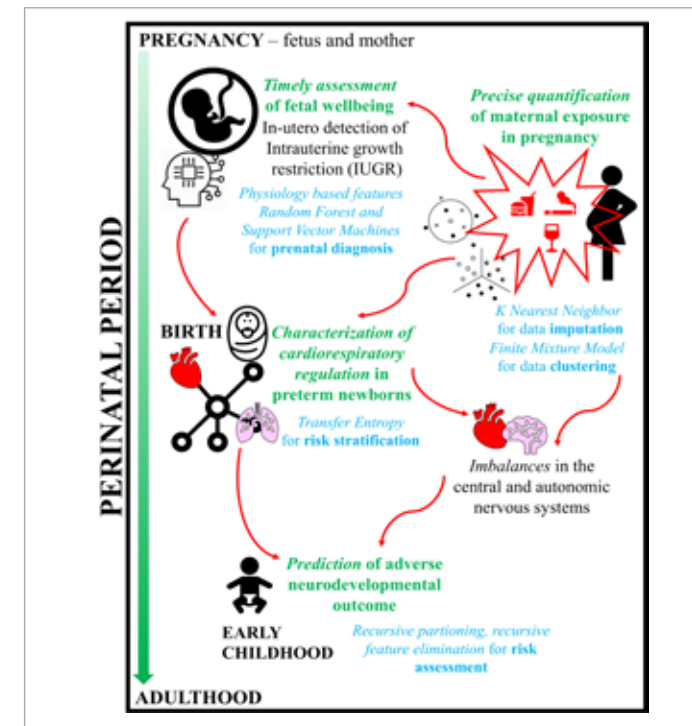

Fig. 1 - Visual roadmap of the proposed machine learning and artificial intelligence sustainable network for perinatal health monitoring trained on heterogenous and longitudinal data.

were unveiled by the utilization of techniques capable of modelling the interaction among subsystems. Lastly, to provide evidence for the sustainability of the proposed ensemble of machine learning and artificial intelligence models we proposed an approach for the prediction of neurodevelopmental outcome in early childhood. The combination of heterogenous data sources weighted and updated based on longitudinal fetal and neonatal physiology, maternal conditions, and diverse environmental conditions is the envisioned approach for a multimodal monitoring framework able to promptly anticipate adverse conditions in the perinatal period. The combination of features collected during pregnancy and at birth such as neonatal EEG, fetal, neonatal, and maternal chart abstractions, home environment, and in-utero substance use was able to successfully predict neurodevelopment outcome data collected at 24-37 years of age. In conclusion, the quantitative framework proposed in this Ph.D. thesis paves the way for a methodological and rigorous sustainable network for perinatal health monitoring trained on heterogenous longitudinal data fusion. The envisioned application is to promote healthy pregnancy, safe childbirth, and reduce adverse outcome by informing monitoring solutions for risk assessment with novel dynamical indicators of perinatal health.

PHYSIOLOGY BASED MACHINE LEARNING AND DATA ANALYTICS FOR PERINATAL MONITORING – A NOVEL FRAMEWORK FOR A COMPREHENSIVE MATERNAL, FETAL, AND NEONATAL PROFILING

Marco Stefanati - Supervisors: José Félix Rodríguez Matas, Yvan Torrente

Tutor: Sara Mantero

Muscular dystrophy is a pathology characterized by the lack of dystrophin in the skeletal muscle that causes progressive weakness and loss of muscle mass. The lack of dystrophin causes an increased frequency of degeneration/regeneration cycles of muscle fibers. This increase in the frequency of degeneration/regeneration cycles leads to incompletely regenerated fibers and gross alterations in the fiber ultrastructure related to the loss of muscle performance. The aim of this thesis is to build a comprehensive chemo-mechanical mathematical model for the dystrophic skeletal muscle of a biophysical basis and simulate the skeletal muscle contraction within the continuum mechanics framework. The main hypothesis behind this model is that alterations at the microscale i.e., fiber ultrastructure, are responsible for the loss of muscle performance, while muscle integrity at the macroscopic scale remains unaltered.

Chapter 1 presents a description of the structure and function of the skeletal muscle, the implications of muscular dystrophy on muscle function focusing on the Duchenne Muscular Dystrophy (DMD), the main progress towards the mathematical modeling of skeletal muscle reported in the literature, and outlines the main objectives of the project.

Chapter 2 develops a three-dimensional (3D) chemo-mechanical mathematical model of dystrophic skeletal muscle. This model is based on stress-strain mechanical data of the muscle and studies of changes in fiber structure and interaction aiming to shade light into the biophysical mechanisms regulating muscle contraction. The main supported hypothesis behind is that the myosin function does not underlie the weakness of the dystrophic muscle and, consequently, the active cross-bridge mechanisms inside myofibrils are not seriously affected by dystrophic disease. So, it is hypothesized that the loss of isometric contraction force and isotonic concentric contraction velocity is a mechanical problem resulting from an increase of muscle matrix stiffness, loss of fibers density, a decrease of lateral transmission force efficiency, non-uniform fiber distribution and myofibrillar structure misalignment. The developed model aims at testing which of these defects are responsible for the dystrophic muscle weakness, i.e. the reduction in isometric force and contraction velocity. The results confirm that the alterations in the fiber microstructure, specifically in the myofibril alignment within the muscle fiber, could be correlated with the reduction of contractile force and shortening velocity in the dystrophic fiber. The resulting model represents an innovative tool for researchers

to predict muscle response under conditions that are not possible to explore in the laboratory and could be an important step in-silico trial to study DMD pathogenesis by providing insights into the underlying mechanisms of muscle response to force and bypassing the use of animal models.

Chapter 3 presents an evolution of the previous model by introducing a more detailed description of the muscle fiber ultrastructure to better explain the disorders associated with the DMD pathogenesis. The results confirm that changes in the muscle microstructure, i.e. the dispersion in myofibrillar orientations, disorders in sarcomere pattern, and fiber branching, have an important impact on the mechanisms regulating the dystrophic biomechanics. In particular, the reduction of active force in the dystrophic muscle is connected to the alteration of the myofibrils alignment within the single fiber, while the reduction of contraction velocity seems to be associated with the chaotic organization of the sarcomeres. So, these microstructural changes, caused by the lack of dystrophin, myofibrils misalignment, and sarcomeres disorganization, together with the myofibrils branching, synchronicity lack of the fibers and increased fibrosis, determine an important reduction of the muscle performance (i.e. isometric

force and contraction velocity) in the dystrophic condition. The resulting model represents an original approach to account for defects in the muscle ultrastructure caused by pathologies as DMD.

Chapter 4 describes the experimental test performed on the healthy and dystrophic diaphragm to study the fiber-ECM interaction by looking into changes in the microstructure of the muscle during monoaxial loading, to formulate a much more accurate model of the tissue and to determine potential mechanisms of damage that compromise muscular functioning in DMD. The muscle was subjected to controlled mechanical deformation using a custom-made device developed and manufactured, and the muscle microstructure was analyzed through a particular microscope that has allowed the visualization of a single fiber. The results of the experimental tests confirm that the kinematics of the fiber-ECM interaction and passive lengthening of the dystrophic muscle are not altered by the pathology. This observation is in agreement with the results of the fiber ultrastructure model (Chapter 3) in which the difference between the healthy and dystrophic muscle lies mainly in the fiber microstructure, in particular in the dispersion of myofibrillar orientation and disorders in sarcomere pattern.

Chapter 5 gives the final discussion and future developments. This chapter also reports the results of an implementation of the proposed skeletal muscle model in the commercial software ABAQUS (Dassault Systèmes®) as a user subroutine for future applications with real muscle geometries.