Al collegio docenti del Dottorato in Medicina Molecolare

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Genetic and acquired cardiomyopathies lead tens of millions of people worldwide to develop heart failure, a severely progressive condition associated with high mortality risk. Despite major advances in cardiovascular medicine, the end-stages of the disease, which are often refractory to medical therapy, require the implantation of ventricular assist devices and/or cardiac transplantation but the existing ones suffer for a number of limitations. In this field, tissue engineering has been broadly explored in the last decade and many materials have proven to be potentially effective to be used to restore heart functionality. Liquid Crystalline Elastomers (LCEs) are new smart materials, able to reversibly deform in response to a given stimulus by generating movement and force. Thanks to these characteristics, LCEs could be exploited to develop artificial contractile tissues, to be potentially employed as a long-lasting therapy for cardiomyopathies.

In the first PhD year, I focused on Duchenne Muscular Dystrophy (DMD), an inherited lethal muscular disease, that is caused by X-linked mutations in the dystrophin gene. DMD gene encodes the full-length dystrophin, a 427kDa protein, that is located between the muscle fiber membrane and the sarcomere and exhibits both structural and regulatory functions. The main functional consequence of the lack of dystrophin is an increased cellular vulnerability to mechanical stress associated with muscle contraction. For a long time, DMD has been considered a skeletal muscle disease but a severe cardiomyopathy affects DMD patients and for most of them the progressive cardiac involvement is limiting for survival. In the field of regenerative therapies, the advent of patient-derived induced pluripotent stem cells (iPSCs), which can be differentiated into functional cardiomyocytes, has opened the possibility to characterize DMD-associated cardiomyopathy. Cardiomyocytes from human iPSCs (hiPSC-CMs) are the most promising human source with preserved genetic background of healthy individuals or patients.

In this work, I used three different hiPSC-CMs: a control line derived from a healthy volunteer and a DMD line obtained from a patient carrying a deletion of exon 50 in the DMD gene. Moreover, I compared these cell lines with a CRISPR-Cas9 genome edited cell line targeting the wild-type locus in the control line. For cardiac differentiation, I performed a monolayer directed differentiation protocol. Spontaneously beating monolayers were usually evident from day 8 while, on day 20 post differentiation, single cells were seeded for further cardiac maturation. However, once differentiated from hiPSCs, cardiomyocytes showed an immature phenotype not comparable to that one of adult cardiomyocytes. In order to overcome the immaturity of hiPSC-CMs function, I cultured hiPSC-CMs in long-term culture on biomimetic substrates, characterized by grooves and ridges that mimic the morphology of cardiac extracellular matrix. The main advantages of these biomimetic substrates are their fabrication, using soft lithography technique and the possibility to modulate substrate stiffness. Simultaneously, we also

performed optical measurements of hiPSC-CMs action potential and calcium transients (Ca-T) to correlate these parameters at specific time points (day 60, 75 and 90 post differentiation), during the maturation process. Moreover, I performed a post-rest potentiation (PRP) protocol to evaluate the sarcoplasmic reticulum (SR) function and calcium contribution. As a proof, the contribution of SR to calcium release was also estimated with Caffeine-induced Ca-T protocol. This work in my first PhD year aimed to evaluate how loss of dystrophin alters calcium handling in DMD-CMs.
In later stages (day 90 vs. day 60), control-CMs revealed increased action potential duration (APD) and Ca-T amplitude and faster Ca-T kinetics. The control cell line also showed increased SR function, as assessed by PRP protocol. Then, comparing DMD vs. control-CMs, DMD cell line revealed a shorter ADP, a lower Ca-T amplitude and faster Ca-T kinetics at all maturation time points.
Now I am currently trying to change substrate conditions, increasing the stiffness of the biomimetic substrates in order to mimic the stiffness variation of the extracellular environment and to evaluate the response in the regulation of calcium homeostasis. As future applications, I will also exploit LCEs as dynamic cell scaffolds to improve hiPSC-CMs maturation, through mechanical stimulus. Among their advantages, these materials combine response to external stimuli and elastic properties and they are able to provide cell alignment. The main idea would be to generate a mechanical stimulus with LCE contractile scaffolds, so to promote hiPSC-CM differentiation and maturation.
To create these dynamic scaffolds, the well-known 3D printing and photo-polymerization techniques will be used to obtain the desired dimension and shape of the contractile patches (CPs) optimizing the alignment techniques to achieve the more convenient contraction pattern.

• Attendance to 13th Meeting of Young Researchers in Physiology, Anacapri (NA) May 10-12 2019, with the lecture *"Loss of dystrophin alters calcium-handling maturation in response to microenvironment in hiPSC-cardiomyocytes from Duchenne Muscular Dystrophy patients"*

- Attendance to lectures at University of Florence:
  - *"Mechanisms of arrhythmias in genetic cardiac diseases"*, R.Coppini; 11/04/2019
  - *"Genetic diseases of the heart"*, Prof. M. Regnier, 15/04/2019

- Attendance to lecture at European Laboratory for Non-Linear Spectroscopy (LENS):
  - *"Computational modelling of human function and drug action: from microstructure to cardiac disease"*, A. Bueno-Orovio; 12/09/2019

- Attendance to Complementary Skills at University of Siena:
  - *"Scientific writing and presentation"*, J.L. Telford; 2-3/09/2019
  - *"Nuovi strumenti per l'analisi della risposta immunitaria alla vaccinazione e all'infezione tramite un approccio di system biology"*, A. Ciabattini, F. Santoro; 5/09/2019
  - *"Comunicare in Ricerca"*, E. Meli, A. AllansDottir; 10-17/09/2019
  - *"Spin-off e start up della ricerca"*, L. Zanni, F. Senatore; 16-25/09/2019
  - *"Creating value from large archive and big data"*, L. Neri; 24/09/2019

- *“La ricerca dell’informazione di ambito scientifico su internet”*, L. Bianciardi; 26/09/2019

• Publication: “*Optical Investigation of Action Potential and Calcium Handling Maturation of hiPSC-Cardiomyocytes on Biomimetic Substrates”*, J.M. Pioner, L. Santini, C. Palandri, D. Martella, F. Lupi, M. Langione, **S. Querceto**, B. Grandinetti, V. Balducci… C. Poggesi, C. Ferrantini and R. Coppini; Int J Mol Sci. 2019 Aug 3; 20(15).