Cardiac modelling of desmin related myofibrillar myopathy in vitro on micropatterned soft hydrogels and automated single cell classification based on subcellular organization by supervised deep learning

V. Batoumeni [1, 2], A. Khedher [1], D. Hoffmann [2], Y. Hovhannisyan [1], N. Wiest-Daesslé [2], P. Sommer [2], C. Migdal [3], P. Joanne [1], L. Brino [2,] O. Agbulut [1]

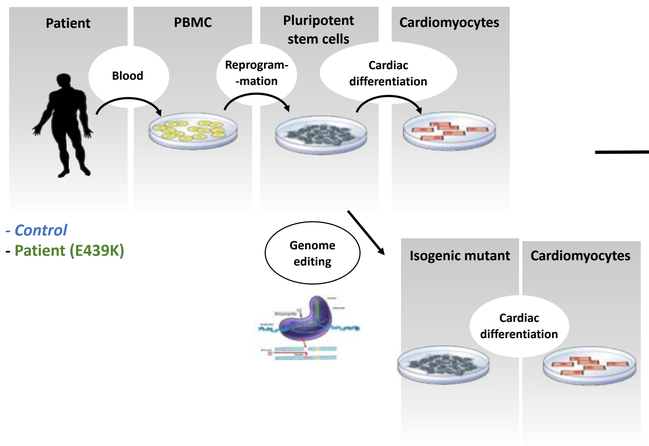
[1]Sorbonne Université, Institut de Biologie Paris-Seine, UMR CNRS 8256 "Biological Adaptation and Ageing", Team INSERM U1164 "Stem cells, cardiovascular pathophysiology and biotherapies", Paris (France), [2]KSILINK - Strasbourg (France), [3]Cell&Soft - Grenoble (France)

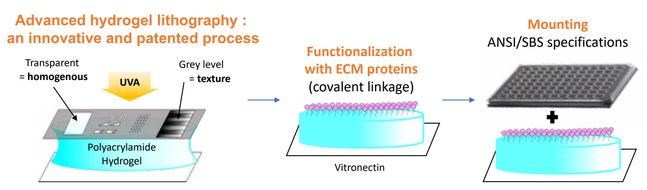
Summary

Myofibrillar Myopathy (MFM) is a rare disease that significantly affects the quality of life and shortens the life span of those affected. Mutations of DES, the gene encoding desmin, the main intermediate filament of muscle cells, is a major cause of MFM[1]. This disease is mainly characterized by a muscular dystrophy but also, in many cases, by the progressive emergence of cardiac dysfunctions evolving frequently from dilated cardiomyopathy (DCM) to heart failure[1]. The following study relied on an in vitro cardiac disease model employing cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs)[2] generated from patients carrying a DES mutation (E439K) and non-affected controls. The hiPSC-CMs were cultured on an innovative soft well plate containing micropatterned vitronectin-coated hydrogels[3,4]. Micropatterns are known to enhance structural maturation of cardiomyocytes. In order to quantify the sarcomere organization in the model[5], an automated deep learning-based morphological analysis software was developed. Here we present our strategy underlying the development of the cardiomyocytes analysis using machine learning and the preliminary results demonstrated the relevance of such approach.

Experimental plan

In vitro Disease Modeling



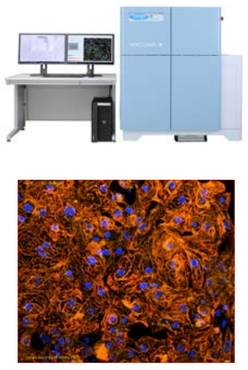


Cardiomyocytes cultured on an innovative soft (kPa Rigidity) well plate containing micropatterned vitronectin-coated hydrogels

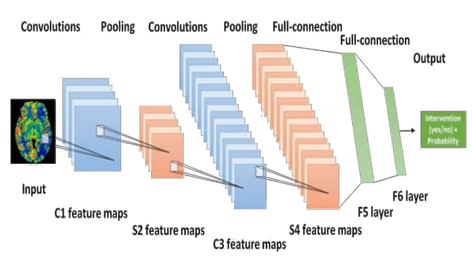
Staining, image acquisition and image analysis

Immunocytostaining
- A-Actinin
- TroponinT
- Nuclei

Automated image acquisition



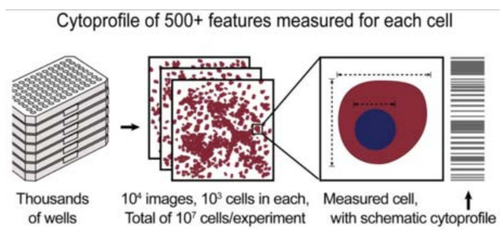
Automated image Analysis

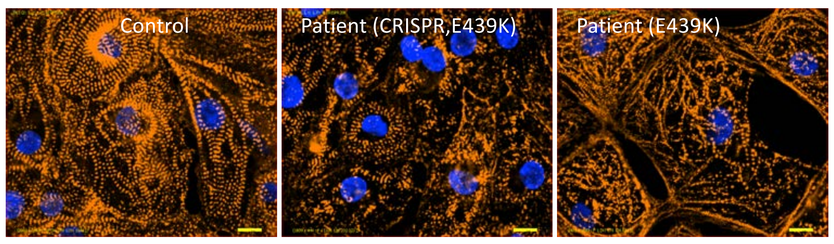


1. Single cell segmentation and manual scoring
2. Training set for machine learning
3. Automated scoring

Automated image analysis strategy

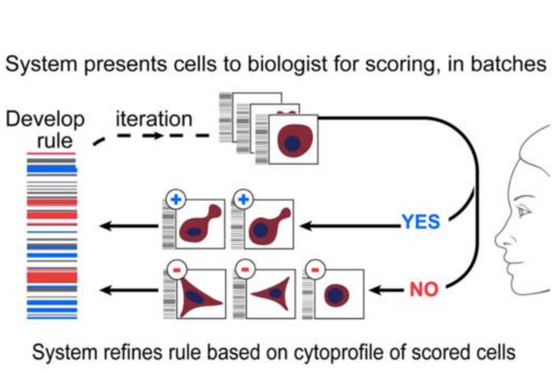
A. Automated cell image processing



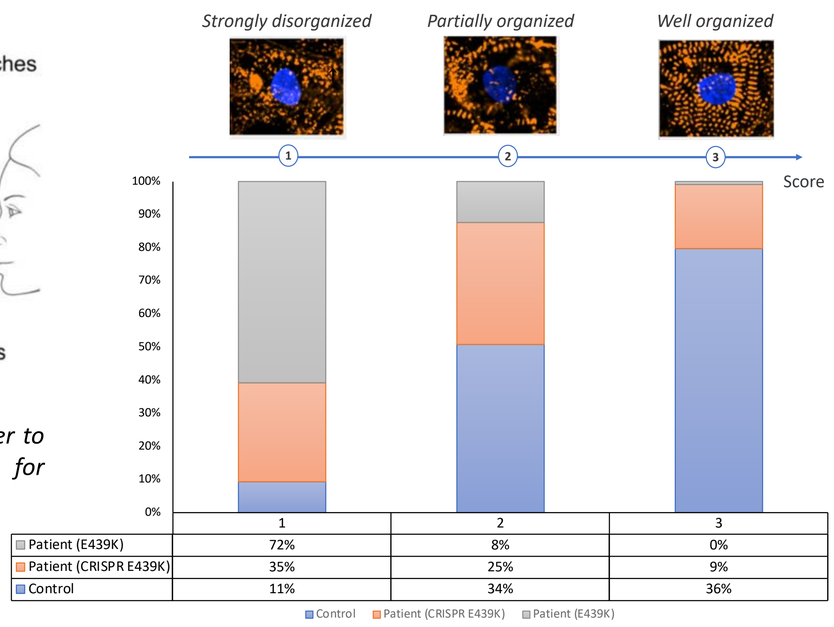


α-actinin Nuclei

B. Manual Scoring



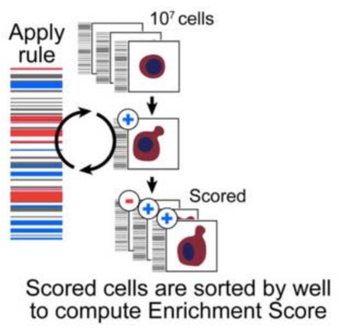
4678 single cells were manually scored in order to implement a machine learning algorithm for automated scoring

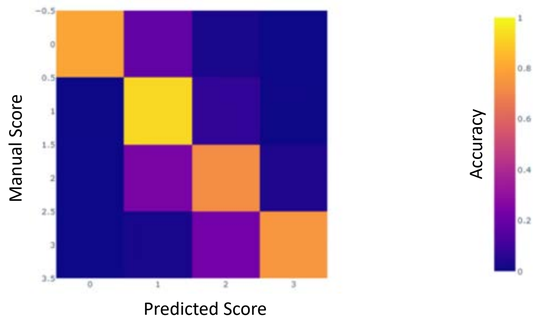


	1	2	3
Patient (E439K)	72%	8%	0%
Patient (CRISPR E439K)	35%	25%	9%
Control	11%	34%	36%

Control Patient (CRISPR E439K) Patient (E439K)

C. Machine learning





Confusion Matrix after a training set on 3678 single cells manually scored and a testing set on 1000 single cells

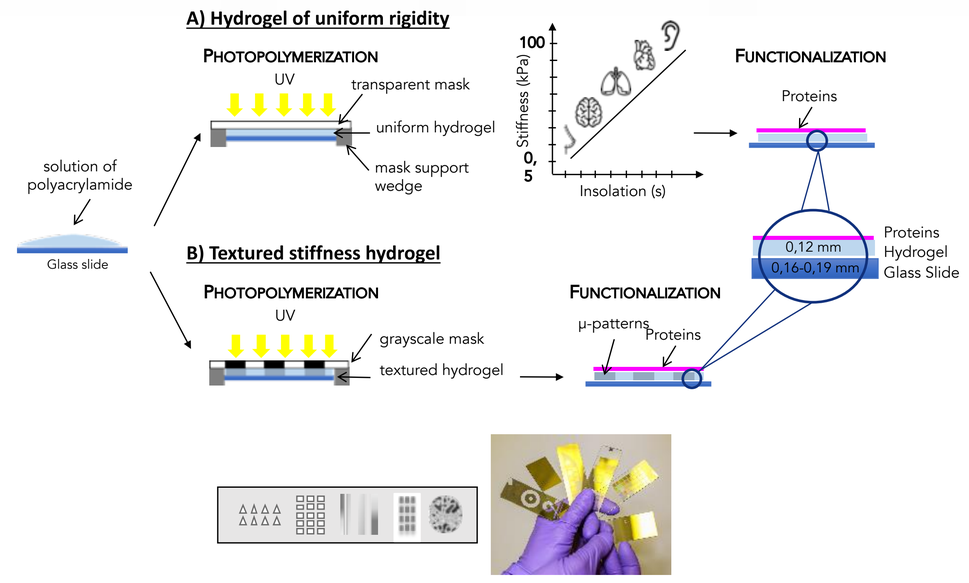
Presentation of the automated image analysis strategy

A. **Automated cell image processing.** The first step consisting of image acquisition and cell segmentation (single cells, identification and cropping around nuclei).

B. **Manual scoring.** The second step is the manual scoring of cardiomyocytes according to their sarcomeric organization performed by a biological expert. Thus, the strongly disorganized cardiomyocytes were scored 1, the partially disorganized cardiomyocytes were scored 2 and the well organized cardiomyocytes were scored 3. Here we can observe the different distribution of cardiomyocytes according to the cardiomyocytes type. Images of cells that were not relevant due to image artifact or signal defect were assigned a score of 0 (not shown in the figure). Thus, the patient cardiomyocytes (E439K) seem to be distributed mainly as strongly disorganized cardiomyocyte (score 1) whereas the control cardiomyocytes are mainly distributed as partially organized or well organized (score 2 and 3). The Patient (CRISPR, E439K) cardiomyocytes appear to have an intermediate distribution.

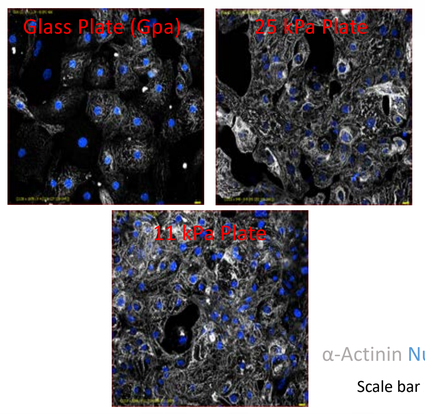
C. **Machine learning**. The last step is the conception of a machine learning algorithm based on artificial intelligence allowing an automated scoring of cardiomyocytes. To do this, a training phase is carried out where many scored cardiomyocytes are presented to the algorithm for its learning. Then, a testing phase consisting in the score prediction for cardiomyocytes that the algorithm has never seen. The validation phase aims at determining the accuracy of the algorithm by comparing for the same cardiomyocyte the score given by a biological expert and the score predicted by the algorithm (Confusion matrix).

Conception of innovative micropatterned and soft plate



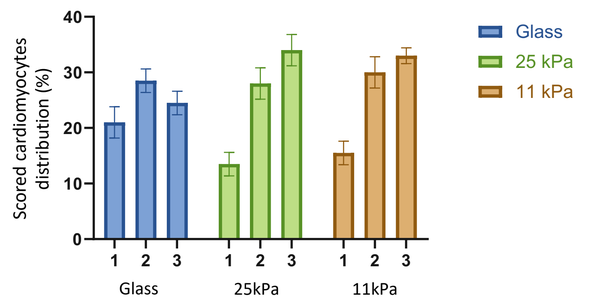
Innovative soft (kPa Rigidity) well plate containing micropatterned vitronectin-coated hydrogels

Distribution of scored cardiomyocytes according to stiffness



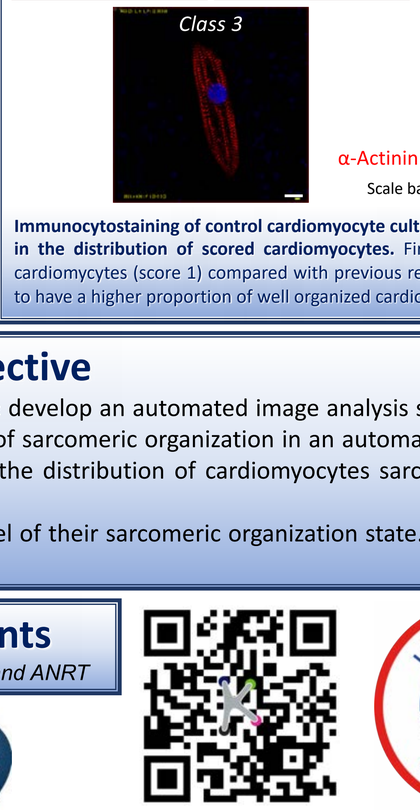
α-Actinin Nuclei

Scale bar 10μm



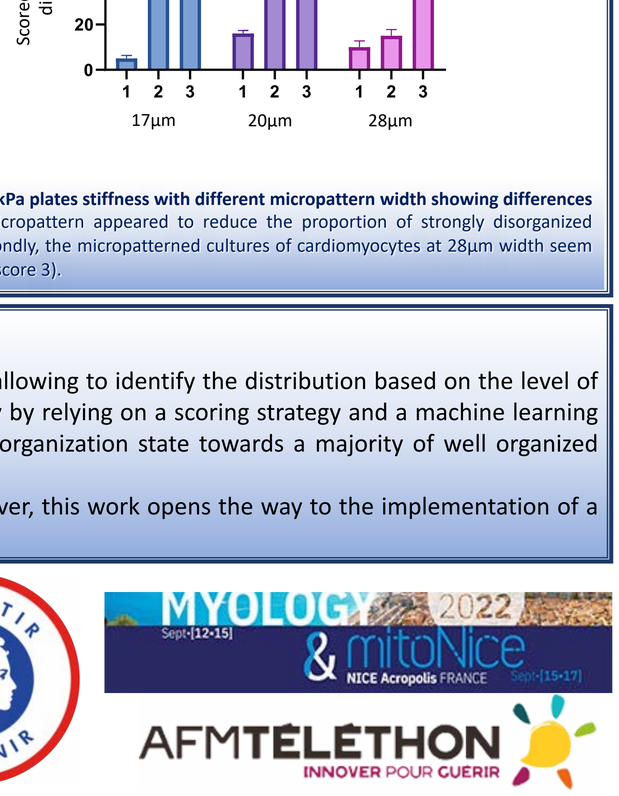
Immunocytostaining of control cardiomyocyte cultured in different stiffness plates showing differences in the distribution of scored cardiomyocytes. These preliminary data suggest that cardiomyocyte cultures on 11 or 25kPa stiffness plates had a lower proportion of strongly disorganized cardiomyocytes (score 1) than in glass plate cultures.

Distribution of scored cardiomyocytes according to micropattern width



α-Actinin Nuclei

Scale bar 10μm



Immunocytostaining of control cardiomyocyte cultured in 11kPa plates stiffness with different micropattern width showing differences in the distribution of scored cardiomyocytes. First, the micropattern appeared to reduce the proportion of strongly disorganized cardiomycytes (score 1) compared with previous results. Secondly, the micropatterned cultures of cardiomyocytes at 28µm width seem to have a higher proportion of well organized cardiomycytes (score 3).

Conclusion and Perspective

Our project consisted in setting up an in vitro culture model of hiPSC-CMs aiming at improving the sarcomeric organization. Then we develop an automated image analysis system allowing to identify the distribution based on the level of their sarcomeric organization. Here, we show that it is possible to analyze images from cardiomyocyte cultures to assess their level of sarcomeric organization in an automated way by relying on a scoring strategy and a machine learning algorithm. In addition, the use of a soft stiffness (11kPa) and micropatterned (Width 28µm) plate seems interesting to improve the distribution of cardiomyocytes sarcomeric organization state towards a majority of well organized cardiomyocytes.

It would be interesting to use the tools developed in this study to characterize hiPSC-CM carrying a DES mutation (E439K) at the level of their sarcomeric organization state. Moreover, this work opens the way to the implementation of a high content screening trial in order to perform drug discovery and identify new therapeutic leads.

References

1. Béhin, A. et al. Myofibrillar myopathies: State of the art, present and future challenges. Rev. Neurol. (Paris). 171, 715–729 (2015).
2. Lian, X. et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions. Nat. Protoc. 8, 162–175 (2013).
3. Paiva, S. et al. Polyacrylamide Hydrogels with Rigidity-Independent Surface Chemistry Show Limited Long-Term Maintenance of Pluripotency of Human Induced Pluripotent Stem Cells on Soft Substrates. ACS Biomater. Sci. Eng. 6, 340–351 (2020).
4. Mgharbel, A. et al. Cells on Hydrogels with Micron-Scaled Stiffness Patterns Demonstrate Local Stiffness Sensing. Nanomaterials 12, 1–17 (2022).
5. Gerbin, K. A. et al. Cell states beyond transcriptomics: Integrating structural organization and gene expression in hiPSC-derived cardiomyocytes. Cell Syst. 12, 670-687.e10 (2021).

Funding and Acknowledgments

This work has been supported by Ksilink, AFM Telethon and ANRT