Development of advanced setups with integrated readouts for evaluation of cardiotoxicity in a heart on chip device

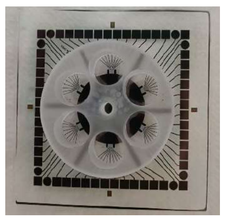
G. Cretti[1], R. Visone[2], U. Kraushaar[3], P. Occhetta[1,2], M. Rasponi[1]
[1] Department of Electronics, Information and Bioengineering, Politecnico di Milano, 20133 Milano, Italy
[2] BiomimX Srl, Viale Decumano 41, 20157 Milano, Italy
[3] NMI Natural and Medical Sciences Institute at the University of Tübingen, 72770 Reutlingen, Germany

SINERGIA Workshop 2023

Introduction

Being inserted in the safety screening field, the aim of the present work was to develop and validate an experimental set-up able to predict functional cardiotoxicity of compounds by exploiting 3D cardiac microtissues engineered in uHeart platforms, benchmarked against state-of-the-art 2D Multi Electrodes Array (MEA) based models. The validation was accomplished integrating a combination of readouts (i.e., motion analysis, calcium imaging, viability assays and field potential recordings).

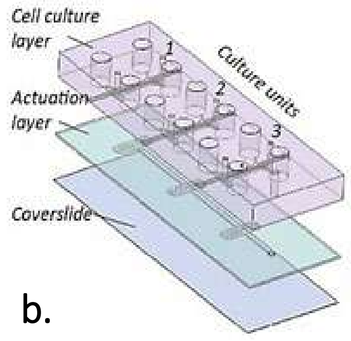
Platforms



a.

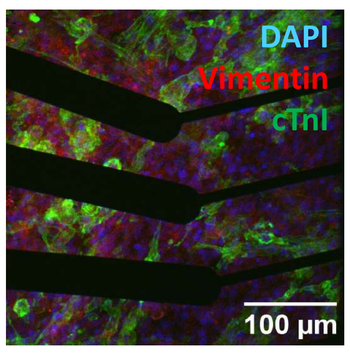
a. MEAs (Multi Channel Systems, Germany) have an arrangement of 60 surface electrodes which are meant for extracellular field potential recording. The cells are seeded on 2D nitrocellulose substrate with a density of 2.5 x 10^4 cells/µl.

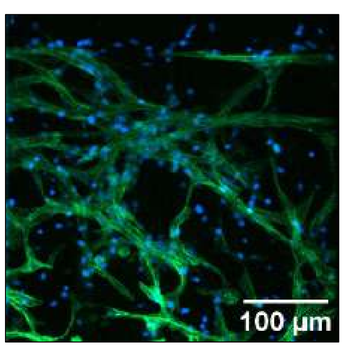
b. uHeart (Biomimx, Italy) platforms consist of three layers: 1) an upper cell culture layer, where the cells are embedded in a fibrin gel and are fed thanks to two lateral channels filled with the medium by four wells per chamber, 2) an intermediate actuation layer which provides a means to mechanically stimulate (i.e., a uniaxial strain of 10%, 1 Hz) the 3D microtissue through a pneumatic actuation system and 3) a bottom coverslip. uHeart platforms can additionally host electrodes to measure the electrical activities of the cells. The seeding density is 10^5 cells/µl embedded in a fibrin gel.



b.

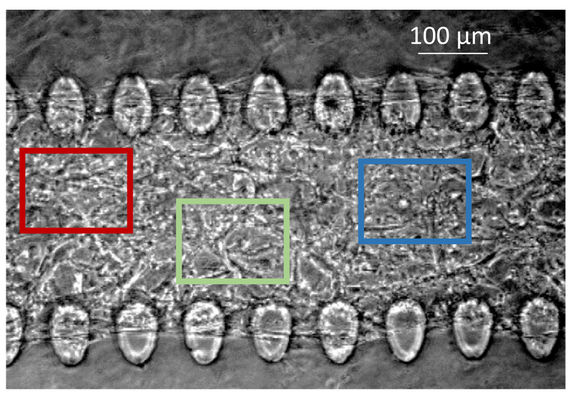
Results

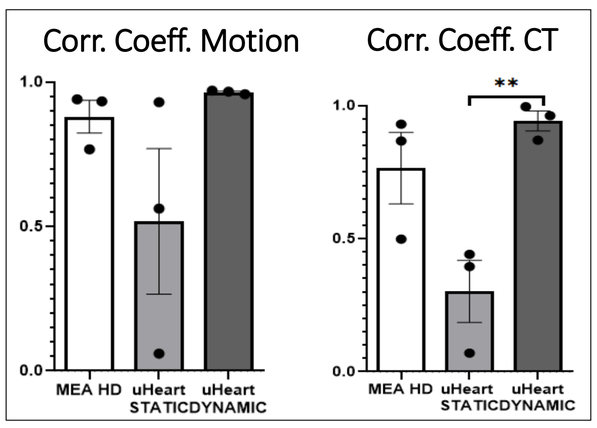




Immunostaining led to discover that the presence of cardiomyocytes (identified by the expression of Troponin I) in the 2D platform was lower compared to uHeart (both static and dynamic), as well as the cardiomyocytes (CMs) in the 3D model were more branched and interconnected.

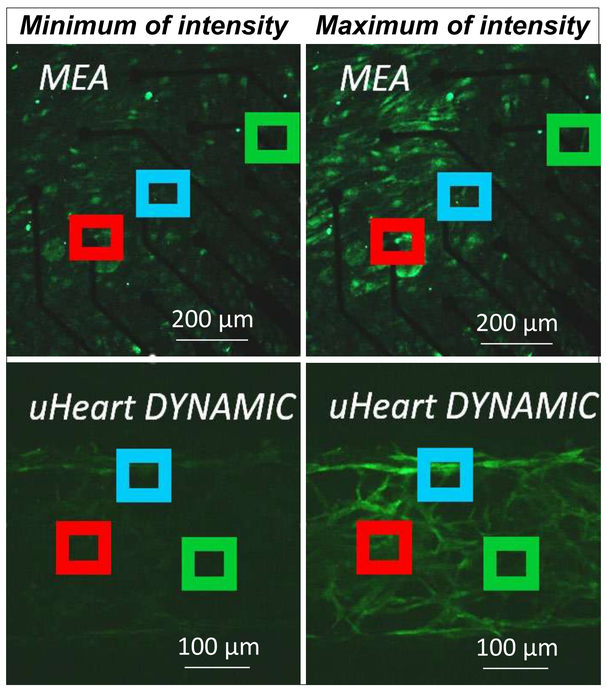
Synchronicity is an important parameter that defines the degree of maturation and the similarity to the primary myocardium. By means of *Musclemotion* software and ROIs function in ImageJ, it was possible to carry out a deeper analysis comparing the simple daily monitoring of cardiomyocytes in different platforms.





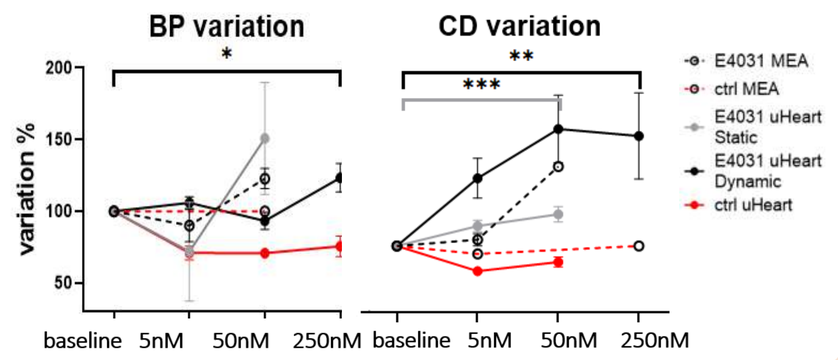
Correlation coefficient related to the beating analyses in different zones of the same sample: the most synchronous microtissues were uHeart dynamic (97%), closely followed by MEA (94.6%). A lower score was obtained by static uHeart, with 56.2%.

The ***calcium transient (CT)*** in each platform was analyzed by means of ImageJ to quantify the synchronicity in the activation of the CMs throughout the construct. The intensity profile was analyzed in three different ROIs. Static uHearts yield a non-synchronous pattern of intensity, while a more synchronous activity emerges from MEA and from dynamic uHeart platforms. Correlation coefficient of the different platforms: MEA 76.5 ± 13.4%, static uHeart 30.3 ± 11.7 % and dynamic uHeart 94.3 ± 3.8% (*p-value = 0.0064).*



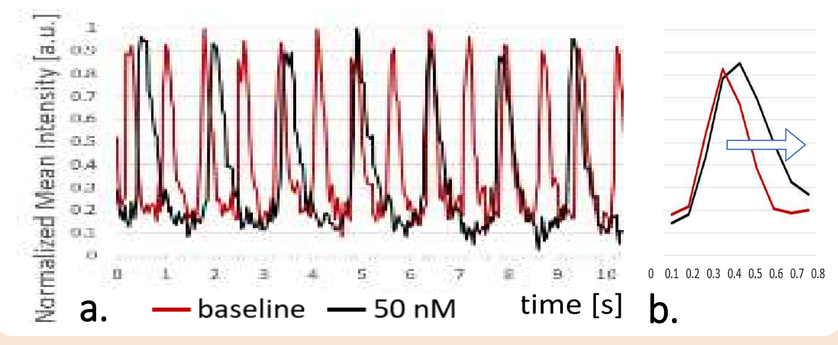
Drug test

The afore mentioned readouts were exploited to assess the effect of E4031 on cardiac microtissue. In all the samples a concentration higher than 5 nM induced an increase in beating period (BP) and contraction duration (CD) with respect to controls without drug, both if analyzed by means of video analysis (above), or through calcium transient (below).



As regards the BP, a statistically significant difference was observed with the highest concentration administered to the dynamic uHeart (*p-value=0.0321).* The CD, instead, was more affected, since both static (*p-value=0.005*) and dynamic (*p-value=0.0022*) uHeart have a clear increase with their highest concentration.

Calcium transient prolongation



Coherently to what observed for the motion analysis, also the calcium transient was affected by the administration of the drug, decreasing the frequency (-47.3±0%) (a) and a prolongation of the duration of the transient (b) by administering 50 nM of the drug. The 250 nM led to stop of the transient, in accordance with what has been seen before in the results concerning the motion analysis.

Conclusions

The experiments highlighted the capability of uHeart platforms to be a very useful and reliable system in achieving more advanced and highly functional cardiac microtissues and in allowing a higher number of combined readouts, potentially evaluated simultaneously, concerning other commercially available systems.

Acknowledgement

The device manufacture was partially performed at Polifab, the micro- and nanofabrication facility of Politecnico di Milano, and partially at Microfluidics and Biomimetic Microsystems Laboratory (Mimic) at Politecnico di Milano.

E-mail: giacomo.cretti@polimi.it

MiMic Lab
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