EPIGENETICS AND CELL CONTROL: CELL GYM ON CHIP

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INTRODUCTION:

Nowadays the worldwide costs related to the healthcare management are continuously rising, so that many efforts have been concentrated in the study of new strategies to extend the human lifespan in the best possible conditions by preserving the wellness. A broad knowledge around the pursuit of an optimum in terms of wellness is still missing. Although genetic approaches are largely exploited *in vitro* to control cell behavior, they are still limited by many concerns [1]. In this scenario, epigenetic manipulation answers many of these issues. Considering that *in vivo* cells are surrounded by a complex and dynamic set of biochemical and biophysical cues, cells can be controlled by these signals, which act alone or synergistically and whose ensemble gives rise to the cell microenvironment [2]. In this direction, literature studies reported that mixtures of bioactive components, in a combination of low doses of single compound, results to be less detrimental with low toxicity level with respect to higher doses-single component [3]. It is therefore paramount to reproduce the effect of different stimuli combinations for cell health maintenance, like in a training program. In this work, the research focused on the design of a platform that can implement these combinations by exploiting the microfluidic and multi-level approach to create a “gym” for the cells. In this view, the first layer is composed by a set of splitting and mixing microfluidic channels to simultaneously obtain four different concentrations of biochemical substances. The second layer is reserved for mechanical stimulation implementation, by means of a deformable PDMS membrane. The device is sterilized and functionalized with fibronectin in order to improve cell adhesion to PDMS deformable membrane. HL-1 cardiomyocytes cell line are then seeded and tested under the action of medium fluxes and mechanical stimulation.

RESULTS AND DISCUSSION:

The designed multi-layer microfluidic device is able to implement gradients of biochemical substances (*Fig.1A*). *Fig.1B* reports a zoomed image of one well of the complete device, in which a chamber for the mechanical deformation of flexible PDMS membrane (*Fig.1C,D*) creates radial deformation field on its central pillar. In fact, as demonstrated in *Fig.2A,B*, the trajectories of fluorescent nanoparticles embedded in the deformable membrane are equally orientated in all the directions of the (x,y) plane. In addition, the device is able to couple biophysical signal to fluxes of substances, as shown in *Fig.2C,* where the flow of a fluorescent dye does not impair the deformability of the PDMS membrane. Cardiomyocyte cells proliferation plot in *Fig.3A* shows that, by functionalizing the PDMS membrane in the microfluidic device with different fibronectin concentrations, cells number already double within 24-48 h when a concentration of 100 µg/mL is used. Coupling of biochemical and biophysical signals have been also demonstrated at the cell level. After the achievement of the steady-state of culture medium flux and mechanical deformation, cell migration has been tracked for 30 min: *Fig.4A,B* show that cell velocity after deformation is higher than migration speed in undeformed configuration, while cell trajectories direction from undeformed to deformed configuration varies from random to radial orientation in the plane, as in the case of fluorescent nanoparticles movement showed in *Fig. 2A,B*. These results demonstrate that HL-1 cells are able to survive, sense and respond to mechanical stimulation while culture medium fluxes are actuated.

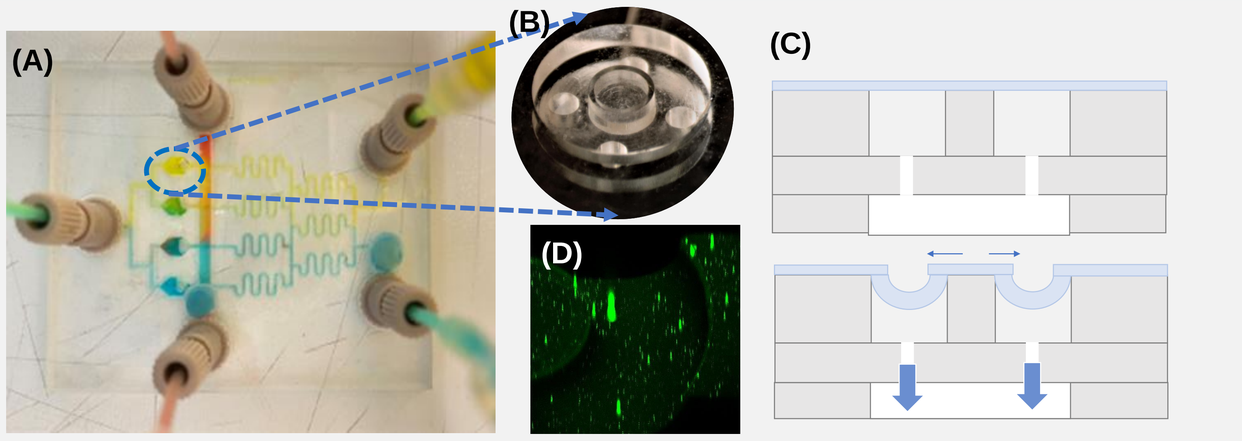

Fig. 1 Microfluidic device for multi-signal implementation. (A) Gradient generator logic. (B) Cell culture well zoom-in highlighting the mechanical stimulation chamber. (C) Integration of PDMS membrane in the microfluidic device and mechanical stimulation working principle. (D) Fluorescent image of PDMS membrane deformation.

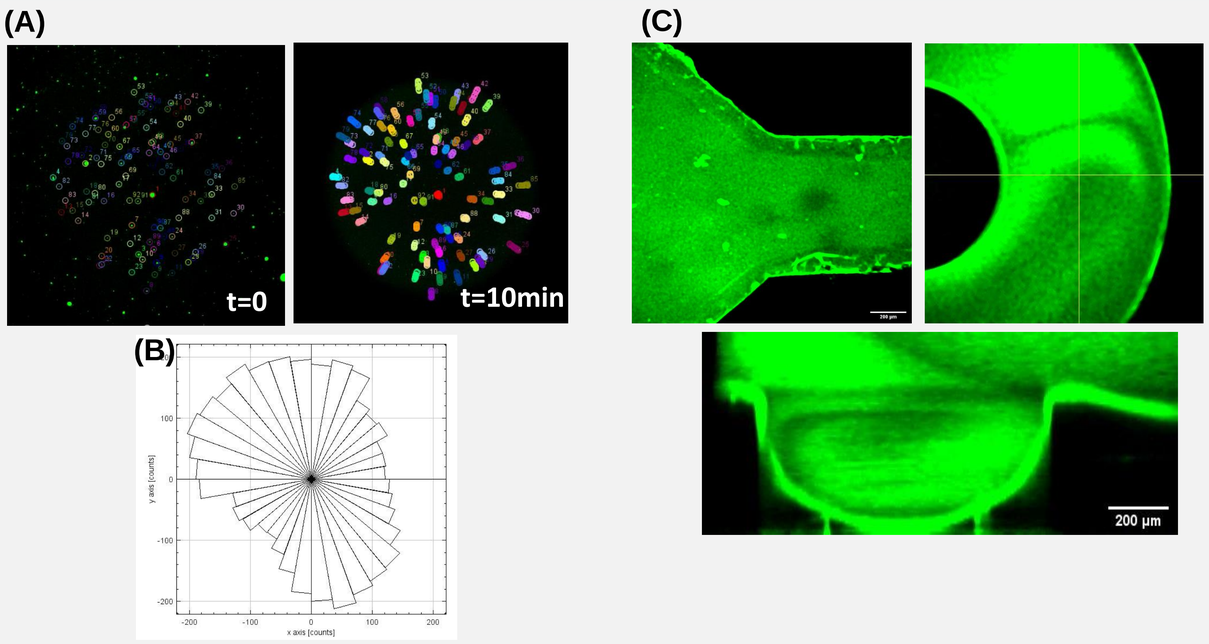

Fig. 2 Microfluidic device testing. (A) Tracking in time of PDMS embedding fluorescent nanoparticles under mechanical stretching. Nanoparticles trajectories demonstrated a radial pattern of displacements. Rose plot of trajectories orientation (B) showed homogenous occurences in all the directions. (C) Coupling of fluid flow did not impair mechanical deformation of the PDMS membrane.

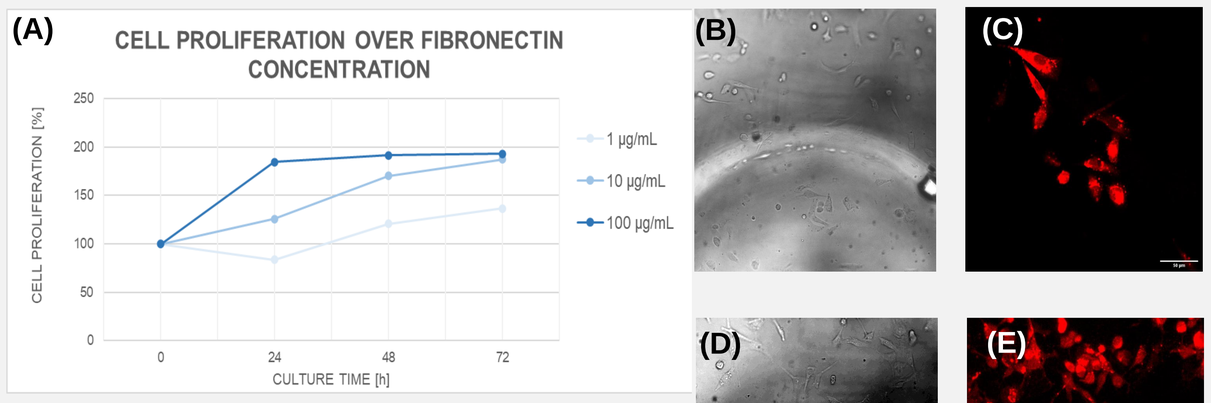

Fig. 3 Microfluidic device flux of culture medium promotes cell survival and proliferation. (A) Cell proliferation has been quantified over 72 h at different fibronectin concentrations, by feeding cells with culture medium flow. Brightfield (B,D) and fluorescent (C,E) images of device-seeded HL-1 cells at 24 h and 72 h, respectivelly, functionalised with 100 µg/mL of fibronectin.

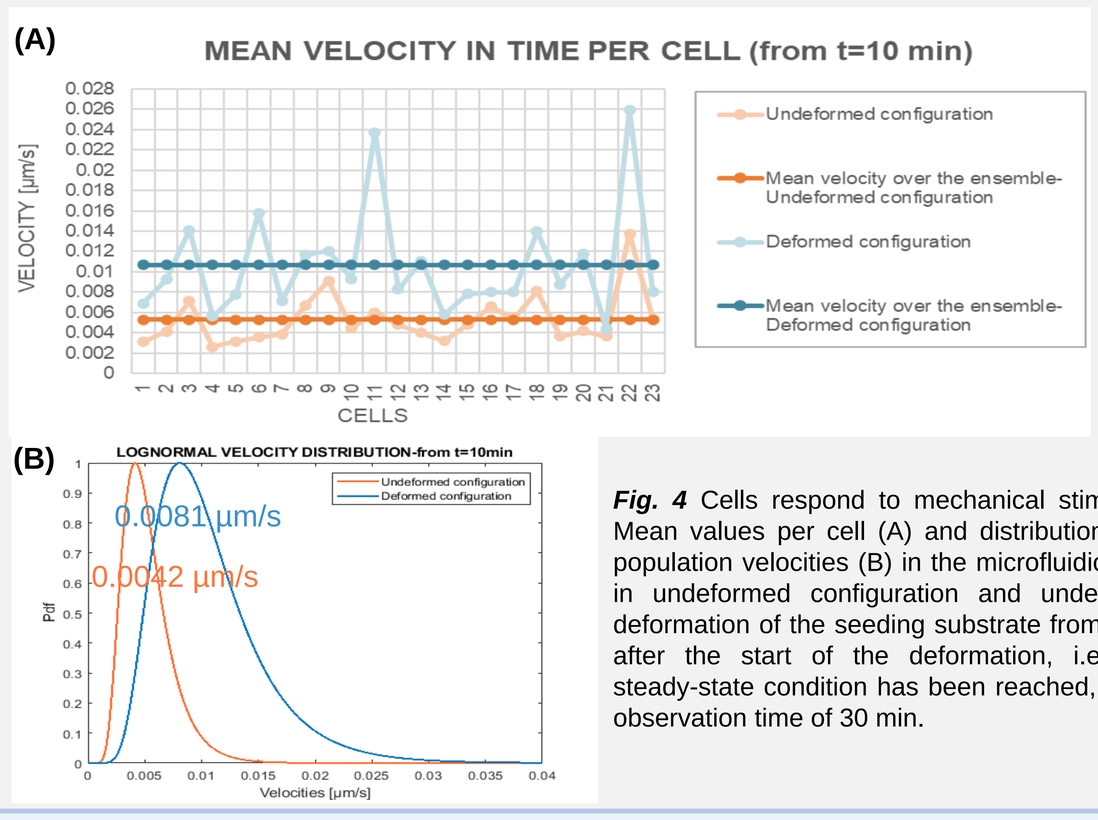

Fig. 4 Cells respond to mechanical stimulation. Mean values per cell (A) and distribution of cell population velocities (B) in the microfluidic device in undeformed configuration and under radial deformation of the seeding substrate from 10 min after the start of the deformation, i.e. when steady-state condition has been reached, with an observation time of 30 min.

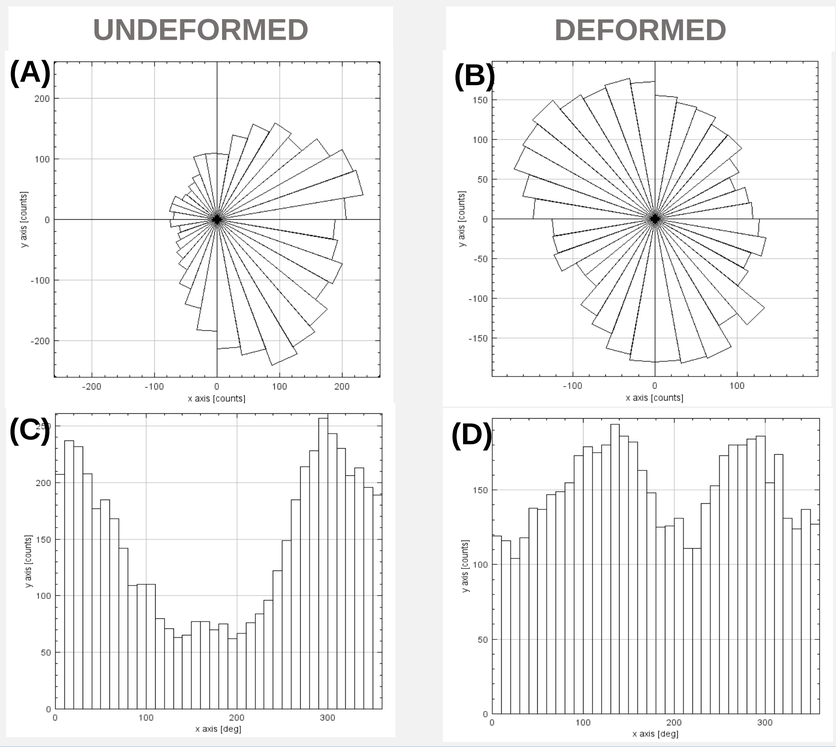

Fig. 5
Cells trajectories in 30 min demonstrate that cells respond to radial mechanical stimulation. Cell trajectories on undeformed PDMS membrane randomly orientate (A,C), while after 10 min of mechanical deformation cells orientate in a radial fashion (B,D).

CONCLUSIONS:

The current study demonstrates how is possible to implement a combination of signals within the same platform by applying microfluidic approaches to cell culture. The biochemical and biophysical signals can be independently and simultaneously implemented and their combination has been shown to do not impair the effect of one signal on another. The biophysical signal is a radial deformation field that is sensed and followed by HL-1 cardiomyocytes also when the flexible substrate reaches its steady-state in deformation. Cells are able to survive and respond to both culture medium fluxes and deformation maintaining their healthy state during the time course of the experiments. All these results pave the way to additional studies on cell wellness: in fact, it is possible to apply all the tested signals by taking advantage of the multi-layered microfluidic platform to induce and study, for instance, the autophagy phenomenon, which is a biological process that cells exploit to preserve themselves from damages induced by aging and/or stressing conditions introduced by variations in their microenvironment balance [4].

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