EPIGENETICS AND CELL CONDITIONING: CELL GYM ON CHIP

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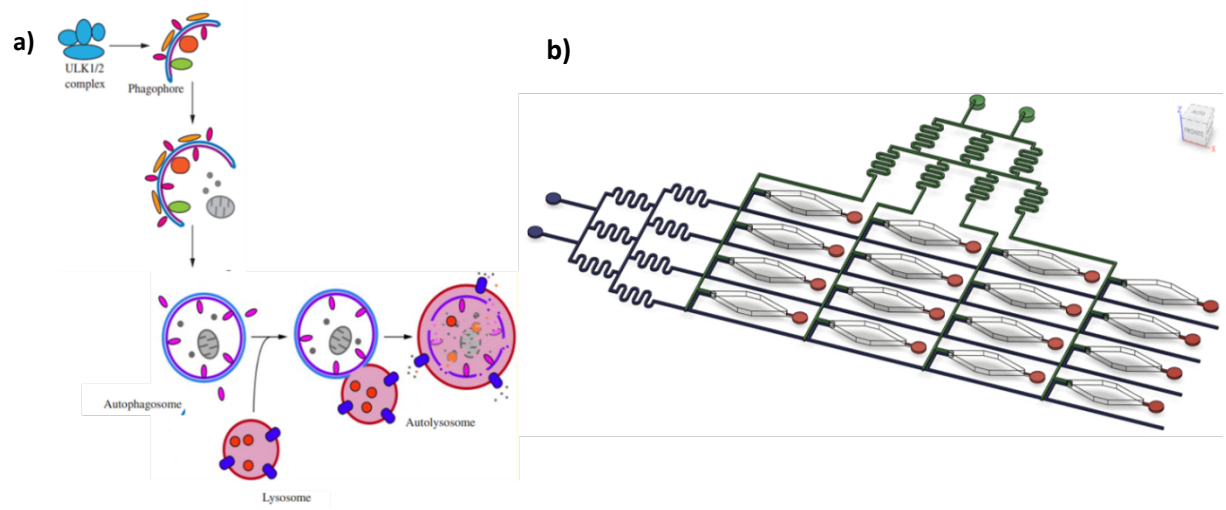
Human wellness currently represents a field of interest in many areas, from the industry to the public healthcare organizations. For these purposes, the attention of the research focuses on the ***bioengineering*** investigation that relates to the design and implementation of systems and technologies looking forward to the preservation and maintenance of human health. A broad knowledge around the pursuit of an optimum in terms of wellness is still missing and further investigations are needed to uncover how to reach this target. A tool largely exploited to characterise the state of the patient is the study of the ***cell*** behaviour.

The awareness according to which cells are controllable and trainable entities is growing between the scientific community, so much that emerging techniques of cell manipulation, like genetic editing, are used to induce a controlled expression of genic materials in the cells. Despite the potential of genetic manipulation, many shortcomings limit their clinical application, due to ethical and safety concerns, as well as to the handling of high costs techniques. For these reasons, the genetic approach is commonly complemented by the ***epigenetic manipulation***, which instead focuses on the influence of the external factors on the cell microenvironment composition.[1] Soluble factors, physical fields, extracellular matrix composition are examples of external determinants of epigenetic pathways and they act alone or synergistically to control cells, inducing changes that do not alter the nucleotide sequence of a gene but only its activity.[2] It is then conceivable that epigenetic signals could be implemented and combined, like in a training program, as factors that are beneficial for cell state. Nevertheless, this is not possible by exploiting the conventional *in vitro* cell culture tools, because of the need of continuum nutrient/conditioning solutions flux and waste clearance, reproducibility of physiological culture conditions and high automation of the processes. These factors lead the research towards the ***microfluidics*** [3], which allows to handle reduced liquid and volumes with consequent decreased analysis time and costs, while the parallelization introduced by microfluidic devices enables better control on the cell conditioning signals in a high-throughput screening of the microenvironment effect on the cell population.

All these concepts will be applied in the submitted PhD work to the design of a ***cell training platform*** that should reveal which conditioning program is necessary to reproduce a healthy cell state by applying a combined action of different signals. The read out of the cell wellness as a result of the training program will be identified in the correct tuning of the ***autophagy***, a conservative process by which cells remove damaged organelles, protein aggregates, excess components, triggered in response to external stressors like pathogens, hypoxia, nutrient deprivation, oxidative stress. The autophagy is a catabolic cellular mechanism whereby cellular contents are sequestered by a double-membrane vesicle termed autophagosome and brought to the lysosome for degradation: this degradation represents a method for cell wellness control.[4] In more details, the introduced PhD work focuses on the development of a multi-array and multi-layered microfluidic circuitry, in a platform that will be able to study the autophagy phenomenon on the treated cell culture. The multi-array approach allows to simultaneously check a variety of cell state within the same platform: cells are cultured in a series of separate chambers and are differently fed by single/combined signals to reproduce a variety of cell responses in terms of autophagy induction. The multi-layer logic requires the design of a device in which different layers separately introduce each microenvironmental stimuli by avoiding any cross-contamination between signals and array chambers. Commonly known techniques of microfluidic device production (microfabrication, micro-milling, replica molding) will be employed to build the overmentioned platform. The exploitation of transparent materials (PMMA, PDMS) will ensure a great accessibility to common microscopy instruments for real-time checking in fluorescence of cell culture response/condition. In each well, cells can be stimulated by biochemical stimuli that will be integrated in the conditioning platform with the aid of microfluidics channels and gradient generators.[5] Similarly biophysical signals can be independently tested or can be coupled to the previously mentioned stimuli and they include variable pH, O_2, CO_2, temperature, which will be included in the platform by using gradient generators of pH/gas mixtures [6] and by locally integrating heating elements.[7] Mechanical stretching will also be counted as conditioning

signal: cells will be stimulated once cultured on thin membranes that will deform under the actuation of vacuum-assisted systems.

The convergence of microfluidics and artificial intelligence represents a powerful tool and it will be implemented in order to manage high throughput large data. The implementation of ***control theory*** plays a crucial role since the combined effect of a plethora of microenvironment signals should be handled by an automated logic. In this way, multiple signals can be tested on the same platform and the control system could help in the modulation of the stimuli level, in response to the read-out of the autophagy cell state in terms of fluorescence detection of the autophagosomes. In light of the design of a new smart microfluidic training platform aimed to the characterization of a defined healthy cell state, this will allow to determine a specific correlation between cell state and microenvironmental signals.



a) Autophagy steps: from the phagophore to the autophagosome formation and final degradation of cell content in the autolysosome (from *An Overview of Autophagy: Morphology, Mechanism, and Regulation. Katherine R. Parzych and Daniel J. Klionsky. ANTIOXIDANTS & REDOX SIGNALING Volume 20, Number 3, 2014*). b) Example of multi-array and multi-layered microfluidic platform with double gradient generation (blue line and green line). Each cell chamber of the array holds one outlet (red) and two inlets (blue and green), placed on three different layers.

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