PHOTO-SWITCHABLE INTERFACES FOR DYNAMIC CELL CULTURING

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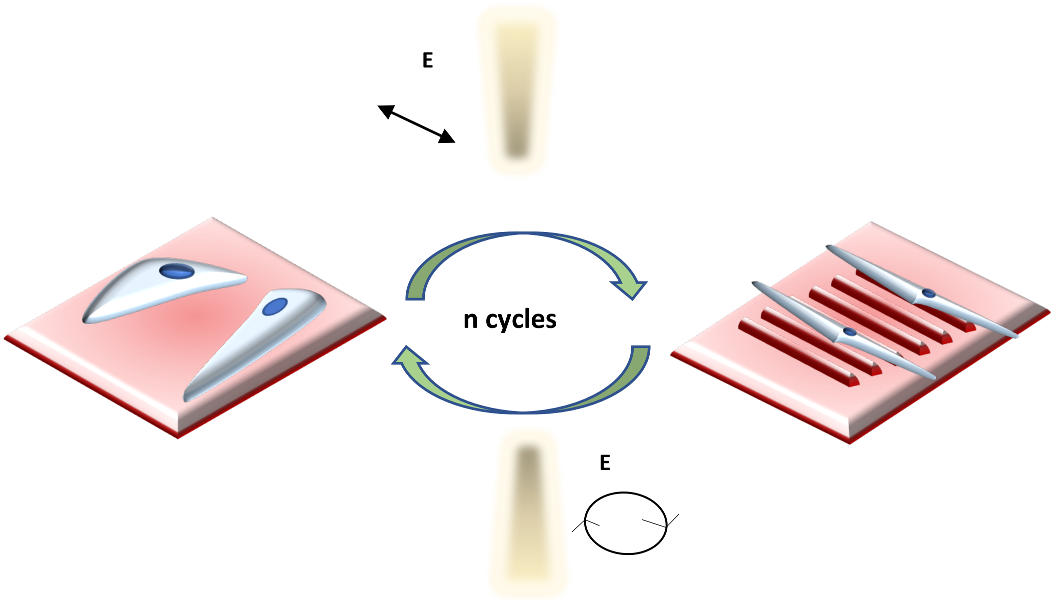
The external cellular environment carries out a crucial role in affecting and dictating cellular functions which are the basis of several biological processes such as morphogenesis, tissue regeneration and repair. The bidirectional communication between cells and the extracellular matrix (ECM) occurs through the transmission of biochemical/biophysical signals at sub-micrometric level that includes sub-cellular cytoskeleton structures and the nucleus, where a series of biological events are activated, eventually affecting the cellular behaviour. In particular, the biophysical signals include both mechanical, such as external forces or forces generated from ECM rigidity, and topographical stimuli. Indeed, the extracellular matrix has an own rigidity and it is also characterized by fibres bundles which provide it with a specific geometry that is involved in shaping tissue architecture and also plays an essential role in cellular and tissue organization**.** Moreover, the ECM is a dynamic entity, continuously undergoing remodelling, assembly and degradation processes during growth and in disease progression. Thus, dynamicity is a fundamental aspect when studying the interaction of cells with the external environment. For this reason, the realization of dynamic bio-interfaces, able to recapitulate reliably the native extracellular environment and to provide precise signals both in space and in time, has been widely spread in the last years, especially for applications in tissue engineering with the aim to design systems for tissue regeneration and repair in the medical field, and for the discovery of new technologies allowing drug screening in the pharmaceutical area. Numerous studies have been done in order to investigate cellular response to external stimuli, but the majority includes the presentation of static signals. In this framework my PhD project is established, which consists in the realization of dynamic bio-interfaces that replicate reliably the native extracellular environment for *in-vitro* cell culturing. In more details, cyclic topographical cues would be presented/removed at specific cell culturing time points and cellular responses would be observed, in terms of morphology, *i.e.* shape change and cytoskeleton reorganization, cytoskeleton and nuclear mechanical properties, and eventually cell fate. The final goal is to control and guide *in-vitro* cell behaviour by providing precise instructions to cells. To this aim, photo-deformable azopolymer material has been chosen to cover glass-bottom petri-dishes as thin film, thus providing switchable platform for cells. Indeed, azobenzene-based materials are constituted by an azo-moiety easily doped or covalently bound to a polymer matrix, which provides them with the ability to change the shape of surface topography in response to light, as a consequence of mass movement triggered by the trans-cis photoisomerization of the azo-moiety. More precisely, when illuminated by UV and visible light, the molecule undergoes a reversible photoisomerization from *trans* to *cis* configuration, which affect local chemical, physical and mechanical properties of the host material. The light-driven isomerization of the azobenzene element acts as a light-to-mechanical energy converter, translating the nanoscopic structural movement of the isomerization azobenzene into macroscopic topographic film modulation in the form of surface relief. The induced surface deformation profile appears dependent on the light intensity and polarization of the applied optical field. In details, by illuminating their surface with a linearly polarized light, a linear pattern, in the form of surface relief gratings (SRG), can be embossed and easily erased with a non-polarized or circularly polarized light. Randomly-distributed surface reliefs can also be obtained under the application of a uniform light intensity and polarization distributions, making these materials highly versatile. Moreover, light-controlled systems are mostly preferred in biological applications because, among all external stimuli used to trigger responsive materials, light provides high spatial/temporal controllability and is not harmful for cells, thus giving the possibility for *in-situ* studies of cellular behaviour. All these properties, together with the feasibility of the system, make azopolymer materials the best candidate for the aim of this project.

The first year of this PhD project was based on the study and characterization of azopolymer-type materials and their optical properties. In more details, laser scanning parameters of a Confocal Laser Scanning Microscope (CLSM) have been optimized in order to emboss and then remove topographical structures in the form of linear pattern able to affect cell morphology. The realized topographical structures were characterized by Atomic Force Microscopy (AFM).

During the second year, the work will be focused on the cellular biophysical characterization in terms of cell morphology and mechanical properties. In particular, in the first period, cell shape, alignment and orientation will be observed by using immunofluorescence confocal techniques. Mechanical properties will be measured by Atomic Force Microscopy (AFM) and multiple particle tracking technique, which provide superficial and internal characterization of cell rigidity, respectively.

Then, during the third and final year, stem cells differentiation to a specific lineage will be observed by several techniques, which allow to observe the activation/deactivation of specific transcription factors during the physical dynamic stimulation.



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