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New light on the interaction between cells and nanopatterned surfaces

A project at the interface of Physics, Biology and Neuroscience sets new perspectives for regenerative medicine.

Contemporary biologists and neuroscientists visualise and study cells with conventional microscopes with a spatial resolution of 1 micron or slightly less. On the other side, molecular and structural biologists reason at a molecular level where events and reactions occur at an Angstrom scale or just above. Therefore, events occurring at a submicron scale and above the single molecule dimension represent a new and unexplored perspective for Biology and Neuroscience. This scale of analysis was chosen as the focus point for the work performed by the NanoScale interdisciplinary research consortium, composed of five major European research centres (SISSA, CNR-INFM, DTU, NMI and ENS) and three small high-tech companies (MCS Gmbh, Promoscience Srl, GVT Srl), coordinated by prof. Vincent Torre from SISSA (Italy).

The project, funded by the European Commission FP7 programme, has produced an improved map of cell interactions with nanostructured substrates thanks to new *Lab-On-Chip* instruments designed within a partnership between academia and industry. These results help the definition of novel cell culture systems, able to influence specific aspects of cell behaviour, with long term perspective of application in regenerative therapy of tissues. The work of the Nanoscale project has been focusing on how a nanopattern design of cell culture substrates influences proliferation and differentiation of cells *in vitro*.

Differentiation is usually achieved by using biochemical factors. However, biochemical induction does not fully prevent the presence of some undifferentiated cells that could become tumorigenic, while the possibility to stimulate and direct neuronal differentiation by cues encoded in the substrate topography opens new perspectives for experimental work. The experiments carried out show that at least one surface topology nanopatterned in soft elastomer substrates has the capability to enhance the differentiation of stem cells into neurons and neural networks: nanopillars. From this point of view, the scientific results that have been obtained by the project are not conclusive. The genetic pathways by which the properties of nanopatterned substrates affect the cell differentiation are still obscure and need to be analysed more in depth.

On the other side, the technological and methodological developments achieved by the Nanoscale team show a big potential for future applications in many research fields.

The partners developed a set of nanotechniques to structure the surface of cell culture substrates with predefined nanopatterns, using X-ray lithography and nanoimprinting. MicroElectrode Arrays (MEAs), which are the most used tool for extracellular in vitro electrophysiology, have been nanopatterned by nanoimprinting and integrated into a complete high throughput prototype setup, which could be commercialised in the future by MultiChannelSystems Gmbh, one of the NanoScale industrial partners.

In order to find the best pattern configuration for growth guidance, researchers also tried to apply mix-and-match approaches of nanoimprinting and conventional photolithography to optimise the substrates pattern design. They found that while both axons and dendrites follow biochemical patterns, etched topographic features seem to be more relevant for axonal guidance. One research team therefore also worked out a novel patterning technique based on nanoimprint lithography and reactive ion etch techniques, that can be applied as a general lithography method alternative to micro-contact print. The resulting high resolution protein patterns are more suited for long term cell culture. Moreover, the project team has developed a novel method for fast 3D imaging of cell interactions in nanostructures, which allows imaging of nanostructures (e.g. silicon nanowires) inside the cells. Finally, a new method for data-driven modelling of cell motility has been established: it can detect the influence of nanostructures on cell motility patterns based on statistical analysis of motility from time lapse movies.

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