Nanostructured substrates to control Embryonic Stem cells differentiation into neuronal lineage.

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The objective of this project was to develop new nanotechnology-based strategies to increase ESCs differentiation into neuronal lineage. In particular it was chosen to investigate a nanostructured physical support for in vitro stem cell culture in which both the nanometrical topography and mechanical properties are well controlled and characterized.

Nanopatterned substrates were designed to have physical properties as close as possible to the in vivo microenvironment where stem cells normally grow and differentiate based on the assumption that mimicking the natural niche equilibrium is of fundamental importance for stem cell fate.

First, an original nanotechnological approach to fabricate the substrates for in vitro neuronal precursors culture was developed.

Secondly the substrate geometrical and mechanical parameters were optimized in order to achieve the maximum differentiation yield of ESCs-derived neuronal precursors (NPs). It was reached a neuronal yield of 74±7% at 48 hours after NPs differentiation induction, which represents the highest yield ever published using nanopatterned substrates with controlled and highthroughput reproducible nanometrical features for cell culture. Moreover it was demonstrated that the mechanical properties of the substrate play a major role with respect to other parameters, such as substrate composition and geometry. A time-dependent analysis showed that the first hours after cell seeding are crucial in the determination of the final differentiation yield.

A further control of ESCs differentiation by manipulating the substrates physical parameters, required a deep understanding of the cell-substrate interaction, therefore it was studied the behavior of neuronal precursors when placed and grown on different artificial substrates using atomic force microscope, scanning electron microscope, and single cell force spectroscopy measurements. The latter lead to a quantification of the forces that develop between neuronal precursors and substrate and provided a clear relationship between adhesion forces and differentiation.

My results suggested the importance of the physical parameter involved in the regulation of the neuronal differentiation and to new guidelines for future applications in regenerative medicine.