Project no. 031847
Project acronym NEURONANO

Project title Towards new generation of neuro-implantable devices: engineering NEUROn/carbon NANOtubes integrated functional units

Instrument Specific Target Research Project

Thematic Priority PRIORITY 3 - NMP-2004-3.4.1.1-1 Towards “converging” technologies

Title of report NEURONANO reporting period P1

Period covered from 1st August 2006 to 31st July 2007

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Start date of project 1st August 2006 *Duration* 36 months

Project coordinator name Laura Ballerini

Project coordinator organisation name University of Trieste (UniTS)

Revision draft 1

Publishable executive summary

The NEURONANO objectives

The NEURONANO network proposes to integrate carbon nanotubes (CNT) with multielectrode array (MEA) technology to develop new generation biochips to help repair damaged central nervous system (CNS) tissues. Our approach is to push ahead the application and functionalization of carbon nanotubes in the CNS by focusing on CNT chemistry, peptide and surface chemistry and two complex networks in cultures: the brain (hippocampus and neocortex networks) and the spinal cord (locomotor networks).
We are specifically interested in understanding how the components underlying electrical activity are organized when implemented with conductive nano-substrates, and how this organization changes in the presence of molecular cues or chronic stimulations.

The research plan has three **major aims**: **i)** to answer fundamental questions about the biophysical interactions between nanomaterials and neurons **ii)** to exploit carbon nanotubes in the presentation of positive and negative cues, thus providing specific molecular environment to favour axonal regeneration and retargeting; **iii)** to develop the characterization of novel MEA/nanotube integrated devices, for multi-site extracellular stimulation and recording.

The **operational goal** of the Consortium is to obtain significant new understanding of:

- neurons and neuronal networks integration on purified and molecularly manipulated carbon nanotube (CNT) substrates
- computational modelling of CNT/neuronal integrated circuits
- molecular modification of CNT
- neurons signalling communication in the presence of CNT
- new CNT/multi electrode array (MEA) devices
- how modified CNT substrates influence and favour inter-neuronal and intra-neuronal signalling
- the identification of new development in the design of MEA and neuro-implantable devices

The Contractors involved

This proposal brings together researchers who are interested in the biophysical basis of neuronal networks dynamics with those interested in nanotechnology, to promote together the development of **nanobiotechnology** applied in the field of nerve tissue engineering.
The consortium gathers four research centres among the most qualified in Europe, one research centre in Israel and one SME (MCS) with a major scientific aim: to engineer a novel generation of neuro-implantable devices based on recent advancements in nanotechnologies. The NEURONANO is based on a large and well-coordinated effort from six different countries (France, Germany, Hungary, Israel, Switzerland and Italy).

Participant name	**Participant organization short name**	**Country**
Laura Ballerini Università degli Studi di Trieste	**UniTS**	Italy
Alberto Bianco Immunologie et Chimie Thérapeutiques Centre National de la Recherche Scientifique Strasbourg	**CNRS-ICT**	France

Katalin Kamaras Research Institute for Solid State Physics and Optics, Hungarian Academy of Sciences, Budapest	**HAS**	Hungary
Henry Markram Brain Mind Institue, Ecole Polytechnique Fédérale de Lausanne	**EPFL**	Switzerland
Shlomo Yitzchaik The Chemistry Institute, The Hebrew Univ of Jerusalem	**HUJI**	Israel
MultiChannelSystems Multi Channel Systems MCS GmbH, Reutlingen	**MCS**	Germany

Co-ordinator contact details

UniTS perform the role of Project Coordinator by carrying out the scientific management with a Scientific Coordinator (Laura Ballerini).

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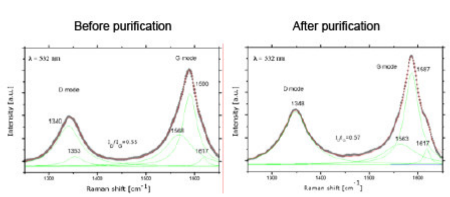
The Work Performed and the Results achieved during the Reporting Period P1

- **Project presentation**

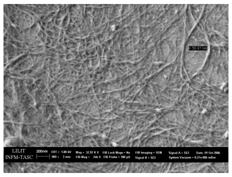
During our first meeting on September 2006, the coordinator presented the NEURONANO project and the newly developed ad hoc web site. The scope of the NEURONANO web site is to create a virtual-bridge between all partners for communicating/exchanging information and scientific results crucial to the project. Across the NEURONANO web pages each partner has a link outlining her/his own contact details, participants, staff involved in the project and facilities. In a "private" section of the web page, which requires a username and password to allow access, WPs, deliverables, team responsibilities or duties and other details of the project are listed. In addition, partners can access to world-wide nanotechnology-related events and files of NEURONANO interests, such as relevant references or proofs or preliminary results, which are uploaded by researchers belonging to the consortium. The web page is also updated whenever results relevant to dissemination priorities will be produced. Project Public **website**: http:// www.neuronano.net

- **Synthesis, purification and characterization of CNT**



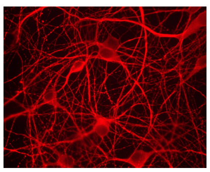
Our premise is that, to learn about the outcome of interfacing cells with chemically modified substrates, we need to provide a system model were long term morphological and physiological tests can be performed while varying the chemical composition of the employed substrates. During the first 12 months of the NEURONANO project, our work has been focused on the development of cultured models (tissues and cells) to evaluate biocompatibility and cellular responses in the presence of CNT substrates under controlled experimental conditions. In the same breath we have performed the planned synthesis and characterization of carbon nanotubes, which were then used for biology tests. The NEURONANO consortium achieved and performed routinely several procedures to test CNT samples, samples were characterized via transmission spectroscopy (FIR, MIR, VIS, UV), Raman spectroscopy and resistivity measurements.

- **Generation and characterization of CNT/modified tissue culturing glass supports**



Upon nanomaterial characterization, we generated CNT/modified tissue culturing glass supports to investigate the biophysical properties of rat cortical neurons (hippocampus) chronically grown on purified CNT for >8 days. CNT-glass support slides together with glass control slides for culturing where characterized by scanning electron microscopy and via electrochemical impedance spectroscopy. We took also advantage of our industrial partner MCS which supplied the technological basics to test the electrical properties of CNTs. Experimental measurements were used to implement the modeling of the "substrate-electrolyte" interface.

- **Biophysical characterization of neurons grown on purified CNT and Model of the interface between nanotube-substrate and neurons**



Hippocampal neurons chronically grown on CNT substrates were monitored via single-cell electrophysiology techniques. We first assessed the changes in network excitability, by monitoring firing activity and synaptic activity under current and voltage clamp conditions, respectively. In a second set of experiments, we specifically addressed the issue of the electrical coupling between CNT and neurons and, based on our experimental results, we propose for the first time a model of neuronal/CNT coupling that will improve the understanding of bio-nanomaterial interactions. Our experimental results, supported by mathematical models to describe the electrical interactions occurring in CNT-neurons hybrid systems, clearly indicate that CNT can directly stimulate brain circuit activity. Noteworthy, this novel finding is also the first report form a European research group that clearly shows how neuronal synaptic pathways chronically grown on CNT substrates can be effectively stimulated via the CNT-layers. Details on:
http://www.neuronano.net/NewsData.aspx?IdNews=21&type=Actual and on:
http://www.nanowerk.com/spotlight/spotid=2177.php

- **Peptide synthesis and characterization and PAN-CNT conjugates functionalization with short peptides**

The peptide sequences for the CNT conjugation were selected on the basis of their neural cell adherence properties and the two initial amino acid sequences reported. For subsequent conjugation to CNT a selective chemical ligation strategy was adopted. For this purpose, a cysteine residue was inserted at the C-terminal part of each peptide. The thiol group of the cysteine allows a selective reaction with CNT that have been modified by introduction of a maleimido moiety. Following our alternative approach, PAN-CNT synthesis was successfully obtained via two methods (chemical and electrochemical) and is ready to be implemented directly to the MEA for interfacing neural networks. The immunological studies with these hybrids have been also started. Once these new materials are proved neurocompatible, studies on the neuroelectronic interface will be initiated.

- **MEA Coating with purified CNT**

MEAs were covered with "positive" (only on the electrodes) or "negative" (everywhere but the electrodes) photo-resist masks. After uniformly layering the CNTs on the MEA-surfaces, we removed the photo-resist masks, thus leaving CNT only on the electrodes or everywhere but the electrodes. In this way, patterned photo-resist can work as a stencil for locally directed coating with CNT. First results with photo-resist covered MEAs showed problems with the deposition of the CNTs. Further tests will be done using different solvents and different photo-resists masks. We also developed a second strategy, based on patterning/deposition of CNTs on MEAs by electrophoresis, which will be applied for structured patterning.

Expected end results

- Assessment of standard CNT devices for biological tests
- Assessment of standard CNT devices for stimulation of neuronal activity

- Assessment of the electrical coupling between nanomaterial and neurons
- Assessment of model system to improve the understanding of CNT/neuronal hybrid systems
- Assessment of peptide synthesis to be combined to CNT functionalization
- Assessment of successful procedures to provide standard CNT-MEA coating

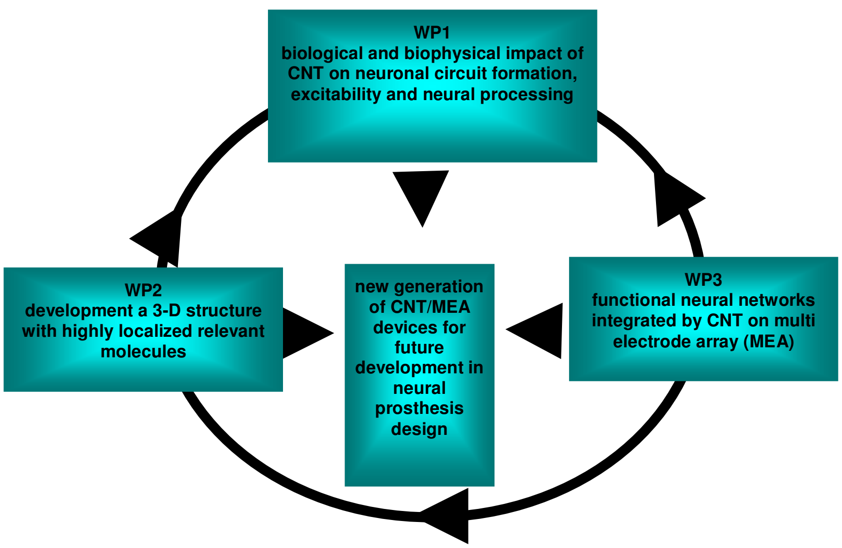
Intentions for use and Impact

The results of NEURONANO will crucially contribute to the development of highly targeted design of CNT-based neural interfaces. Exploiting nanotechnology applications to the nervous system will have a tremendous impact in the future developments of microsystems for neural prosthetics as well as immediate benefits for basic research. Our research activity anticipate technological needs and substantially reinforce and integrate the strong position of Europe in *Nanoscience* by proposing the scientific outcome of a novel and real competitive consortium in the field of nanotechnologies applied to neuroscience.

Plan for using dissemination and knowledge

-dissemination through web site (information for the general public, the press, interested scientists and internal web site for NEURONANO scientists)
-dissemination through thematic research seminars
-scientific publication in peer review journals (6 publications during the first year)
-scientific conferences (participation at international conferences and special sessions devoted to NEURONANO topics at international conferences)

Diagram illustrating the work of the project, project logo and website



http://www.neuronano.net

Final conclusions

The NEURONANO project has made a good start, with an excellent integration among different partners. Several new collaborations have been promoted by the NEURONANO plan during this first year, leading to the exchange of knowledge, personnel, materials and laboratory techniques. The NEURONANO consortium is actually looking at research and innovation to be planned based on and beyond the NEURONANO itself.