Publishable executive summary

A European Research Team led by researchers from the Medical Science laboratory – group of nanotechnology of the Scuola Superiore Sant'Anna in Italy commenced work in December 2006 on the important framework-6 EC project 'NINIVE' (NMP4-CT-2006-033378). The multidisciplinary team involved in the project included scientists from the Center for Drug Delivery Research of the ULSOF (UK), the MPI-FKF fuer Festkoerperforschung (D), Thales research & technology (F), the Institute of Neuroscience of CNR (I) and Nanothinx S.A. (GR).

The main objective of the NINIVE project (*http://www.ninive-project.org/*) is the development of a non-viral vector for a safe and efficient gene transfection and targeted drug delivery. The system proposed by NINIVE is based on use of carbon nanotubes (CNTs) which consist exclusively of carbon atoms arranged in a series of condensed benzene rings rolled-up to form single or multi-walled tubular structures. This novel nanomaterial belongs to the family of fullerenes, an allotropic form of carbon. CNTs have nanometric dimensions and unique physicochemical properties which make CNTs unique materials with several potential applications especially in the biomedical field. Specifically, in the NINIVE project CNTs act as transporters of genes in addition to being functionalized with appropriate ligands to enable specific binding to receptors over-expressed by the cells targeted for transfection. A solution containing myriads of these coated and functionalized nano-vectors is administered by local injection in the target tissue. These nano vectors home on and bind to the intended target cells. In the NINIVE project, two strategies of cell transfection are investigated: (i) cellular up-take of the gene-carrying nano-vectors by endocytosis and (ii) transfer of genes from the nano-vectors to the recipient target cells via electroporation (i.e., permeabilization of cell membrane by application of short-duration electric pulses). With this mechanism, cell permeabilization is induced by CNTs on exposure to external electromagnetic fields by exploiting their nanotransducers properties.
Following confirmation of the proof of efficacy by *in-vitro* studies, the NINIVE system as a platform for targeted gene delivery now requires validation by *in vivo* pre-clinical studies, followed by phase I clinical trials involving patients with a specific neurological disorder. This ambitious project developed by the multidisciplinary NINIVE team covering a broad range of scientific expertise in the fields of nano-biotechnologies, communication technologies and neuroscience has achieved all the objectives outlined in the proposal including efficacy of the NINIVE vector as transporter of genes and its biocompatibility.

All the milestones planned in the project have been succesfully concluded.

The Group of Medical and Ethical Experts (GMEE) was constituted. The experts are Dr. Caterina Cinti, Dr. David Klatzmann, Dr. Luis Mir, Dr. Ulrich Lauer, Dr. François Lachapelle. They were engaged to overview the project and provide advisements. For the expert recruitment, the Consortium followed strictly the EC guidelines.

The State of the Art (SoA) was deeply analysed by all partners. As results of this deep research, we concluded that nowadays there are thousands of publications in the literature concerning the use of carbon nanotubes in biomedicine, and many of them concern the CNT ability to translocate through plasma membranes, allowing their use for the delivery of therapeutically active molecules. Despite this strong interest, there are very few examples of exploitation of the CNT unique electrical, optical, thermal, and spectroscopic properties in a biological context. We concluded that the real breakdown of NINIVE project over the current research is the discovery, not yet achieved, of a new methodology, which exploits CNT physical properties to perform an enhanced and effective *in vivo* gene and drug therapy and no competitors have been identified yet. Concerning the biocompatibility studies, the analysis of the existing literature pointed out the need of procedure standardization for biological testing. Also nanotube characterization should be accurate, as biocompatibility results are closely dependent from the CNT properties, in particular length.
The medical requirements and specifications of gene therapy via electroporation and technical specifications of NINIVE tool for gene therapy have been identified by the Consortium and described in the dedicated deliverable. The results achieved in this task came out from the exchange of know-how among the partners

with background in nanotechnology (SSSA, NTX and Thales), nanoscience (CDDR, MPI), and biology (CNR). This work provided the necessary input to propose the design of the NINIVE vector. In parallel, CNR provided the model of brain disease to try an experimental gene therapy in rodents using the NINIVE vectors.

The aspects related to how NINIVE nanotransducers are envisaged to work, such as the diffusion within the target tissue, the mechanisms of targeted cell binding, cellular uptake via endocytosis and via electroporation were investigated by SSSA, in order to identify base models by using both analytical and finite-element modelling. The architecture of the NINIVE vector was proposed. The basic idea is to cover the nanotube surface with charged functional groups (-NH3+) able to bind DNA. These charged groups are provided by a low density (25 kDa) PEI covalently binded to the weakly oxidised nanotube surface (ldPEI-CNT). The other molecules (i.e., fluorescent molecule for nanotube tracking and ligand for cell binding) are attached to the nanotube by covalent binding to the amino groups. The final molecules chosen for this application were a chemokine and alexa fluor. Several recent evidences suggest that chemokines have a neurotransmitter/neuromodulatory role on brain functions similar to several neuropeptides. In the stroke (animal model for the NINIVE study), among the molecules that allow trophic support to the neurons in the penumbra surrounding the infarct area, the chemokine CXCL12 (previously known as SDF-1) is expressed. This chemokine is quickly internalised by the neurons that express its receptor CXCR4. So, we believe that NINIVE vectors can be functionalised with this small (10 KDa) protein to specifically target the neurons located in the penumbra. Moreover, the internalization of the CXCL12 receptor, CXCR4, could help the localisation of the nanotubes.
Finally alexa-ldPEI-CNTs were produced which are a valid tool to follow the pathway and the diffusion of the nanotubes after injection. The localisation of these fluorescent nanotubes was followed *in vivo* by fluorescent/confocal microscopy.
All the methods, methodologies, and technologies required for the development and the characterization of the vector were identified and proved to be effective. The main out coming of all this activity was the development of the Standard Operating Procedures (SOP). These SOPs represent the summary and the conclusion of the first year of experimental activity performed in the project. They establish the Material & Methods of the Project and assure the homogeneity of protocols used by partners in their experimental activity.

Nanothinx (NTX) produced carbon nanotubes (CNTs) samples and delivered them to the other partners for testing. The first set of samples delivered were products already commercialised by NTX. The aim of this phase was to allow the other partners to test different samples in order to provide the specifications and the requirements for *ad hoc* nanotubes suitable for NINIVE application. The higher purity of the carbon nanotubes were the first specification dictated by the Consortium. Therefore, the second and third delivery concerned purer CNTs, produced and characterized *ad hoc* for the project (MWCNT 99%). Nanothinx tried to develop a material with the highest purity as possible, whereas at the same time exhibited uniformity concerning its dimensions & properties. This sample was tested from the partners with expertise in biomedical fields which considered it satisfying. The fourth delivery of Nanothinx concerned higher oxidised nanotubes to be used by MPI for polymer wrapping via covalent approach. Nanothinx spended some effort on the synthesis of arrays of multi-wall carbon nanotubes (vertically aligned MWCNT, VAMWCNT). The use of these VAMWCNT could bring additional advantages. VAMWCNT could be useful for proving new functionalization strategies (such as nanotube tip functionalization). The last delivery of NTX concerned the synthesis of MWCNT 98.5% in purity. These nanotubes were produced at a production cost which is significantly lower than that of MWCNTs 99% but the cytotoxicity was negligible as well as 99% pure MWCNTs.
All delivered samples were characterised by NTX in terms of diameter (TEM, SEM, AFM), length (SEM), number of layers (TEM), carbon purity (TGA, SEM, EDX), type and amount of metal particles (TGA, ICP, EDX) and amorphous carbon (TGA, Raman, XRD).

For the development of the DNA:polymer-coated CNT conjugates, the CNTs have been coated with positively charged polymers. Procedures for coating MWCNTs with different positively charged polymers were implemented, based on non-covalent and covalent binding. Many polymers were tested: Chitosan, Glycol-modified chitosan, Betaine-modified chitosan, Polydopamine, Polylisine, Polyallylamine (PAA),

Dendrimers, high density PEI (75 kDa) and low density PEI (25 kDa). The efficiency of DNA binding was evaluated via electrophoresys, z-potential and dynamic light scattering analysis. At the end of this work, we can conclude that oxidized-multiwalled carbon nanotubes covalently wrapped with low density polyethyleneimine (ldPEI; Mw ~25,000) are optimal vectors which satisfy the requirements of 1) DNA binding, 2) negligible citotoxicity and 3) capability of gene trasfection. Additionally, the addition of a hydrophilic shielding component polyethylene glycol (PEG) to the complex ldPEI-CNT:DNA provided increased stability and lower cytotoxity. The evaluation of the ability of PEI-MWNTs, PAA-MWNTs and PEI/PAA-MWNTs to induce gene expression was evaluated after incubation with A549 cell lines. All these polymer- MWNTs showed to enhance gene expression in comparison to naked pDNA. The enhancement in gene expression was maximum for PEI/PAA-MWNTs at 10:1 mass ratio and PEI -MWNTs at 30:1 mass ratio. Enhanced gene transfer compared to naked DNA was also proved on primary neural cell cultures.

The CNTs coated with low density polyethyleneimine (25 kDa) have been functionalised with cell ligands and fluorescent molecules (for in-vivo tracking) and complexed with DNA. Specifically chemochine-ldPEI-CNT:pDNA and alexa-ldPEI-CNT:pDNA were produced. All the functionalised nanotubes maintain the capability to bind the DNA. Additionally, in chemochine-ldPEI-CNT:pDNA, we proved:

- the chemochine covalently binded to ldPEI-CNT maintain its biological activity;
- these vectors exhibit increased selectivity towards the neuronal cell type.

Alexa-ldPEI-MWCNTs have been injected into the cerebrocortical parenchyma. Three days and 18 days after the injection, CNT localization was investigated by confocal microscopy. We observed a certain diffusion of red staining around the injection site. A closer view of the cortical neurons by confocal microscopy revealed a specific intracellular localization of the CNTs.
We demonstrated that the vertical aligned carbon nanotubes (VACNTs) grown on Si wafers can be successfully functionalized with different biological molecules or polymers directly on the substrate. Oxygen plasma treatment was found to be an effective tool for VACNTs functionalization. The plasma treatment method has a grate potential for producing materials with controllable physico-chemical properties. Such an approach significantly simplifies the synthesis and purification issues. After modification the nanotubes can be mechanically dissected without remarkable breakages.

In vitro cytocompatibility of all samples released by NTX was assessed by CDDR, CNR and SSSA and experimental data allowed to exclude significant cytotoxicity.
First bare nanotubes (dispersed with the aid of a surfactant, Pluronic F127) were tested. Long-term toxicity studies have been performed with different CNTs and cell viability has been evaluated on SH-SY5Y cell line by using four independent assays. After 2 weeks of continuous incubation, the WST-1 cell proliferation assay revealed a very good viability for cell treated with MWCNT 99% (~90%) and MWCNT 97% (~87%), comparable to the control PF127 (~88%); a little decrease in cell viability (~81%) was observed for cell incubated with MWCNTs 97% acid treated (HNO_3). No ROS was detected within and no apoptosis was induced within 72 hours.
Toxicity studies on primary cell culture revealed that Pluronic F-127 induced apoptotic death but the death was avoided in presence of Pluronic-MWCNT at micrograms per millilitres concentrations.
Concerning *in-vivo* studies, little is known in literature about toxicity of carbon nanotubes and of their dispersion factors in the brain. We showed for the first time that a solution of MWCNTs coated with Pluronic F-127 surfactant can be injected in the mouse cerebral cortex without causing degeneration of the neurons surrounding the site injection.
Internalization assays were performed via TEM analysis and fluorescent labelling. We observed that nanotube uptake by living cells occurs through an energy-independent non-endocytotic pathway. We tested different nanotube samples and we found difference in the sample uptake which could be attributed to the differences in nanotube length: experimental results show that sub-1 μm MWCNTs are easily accumulated within cells.
Second, the ldPEI-CNTs (final vectors) were tested. Experimental data indicated that SH-SY5Y cells exposed in culture for 3 days to ldPEI-CNT at the concentration of 5μg/ml maintained their growth rate and showed normal mitochondrial activity and metabolism when compared to controls. In addition we did not observe any apoptotic cells and reactive oxygen species (ROS) in cells treated with ldPEI-CNTs for 3 days.

Acute toxicity of ldPEI-CNTs:pDNA complexes was tested in vitro using A549 cell line, and the percentage viability using MTT or LDH assay was reported after 4 hrs, followed by a recovery period of 18hrs. The experimental results revealed that the vectors were not toxic at ldPEI-CNT:pDNA mass ratios up to 1:5.
The vectors were thus tested on primary mixed neuronal cultures. The staining of the cells did not show apoptotic features either in presence or absence of CNTs. Treatments for 24h at 37°C of control neurons and glia in presence of 1 or 2.5 μg/ml ldPEI-MWCNTs, with or without 2.5 to 5 μg/ml DNA respectively, did not show statistical difference. The total number of apoptotic cells in each sample was around 20%. No statistical significance was found among the different treatments.
To assess whether variations in gene expression was directly associated with various degrees in pDNA uptake after complexation with various polymers or polymer-CNT vectors, we performed trafficking experiments where fluorescein labelled DNA was complexed with the vectors and incubated with A549 cells for 4 h. The results suggested the endosomal pathways as a route of internalization of polymer complexes. On the other hand, polymer-CNTs:pDNA were not co-localized with endosomes, but more aggregate complexes were observed in the cytoplasm. Also, the uptake process seems to occur at a slower rate compared to the polymer complexes.

The performances of CNTs as receiver antenna were studied by Thales as a function of CNT features (i.e. diameter, length and resistance) and of the transmission frequencies. Power transmission between the transmitter and the CNT like-antenna were modelled and designed both in air and water enviroment. Finite element modeling demonstrates the ability of a CNT to work as antenna. The theorem of antennas and simulation predicts that multiwall nanotube (MWCNT) dipole antennas should resemble thin-wire dipole antennas for nanotube EM-wave propagation at or near the free-space velocity of light. The following step of this study was to insert in the model the real input values (diameter, length, conductivity), directly measured on the nanotubes used in the project, and thus obtain the output parameter to be used for the final realization of the system. To do this, a deep characterization was also performed at Thales lab, in particular concerning the nanotube morphology and the other issues relevant for the design of nanotube like antenna. SEM analysis allowed to identify the nanotubes morphology (diameter, length, etc.). High resolution TEM and electron diffraction experiments revealed the nanotube structure (hollow core, polygonized texture, graphene sheet distance, defects, chirality and conductivity). On the basis of the predictions from the model, we fixed the working wavelenght and frequency of the radiation. The design of the circuit and the layout of the experimental bench for electroporation was thus provided.
Preliminary finite element modeling (FEM) was performed to simulatethe in-vivo experiments on wireless transfection in mice. A 3-D electromagnetic simulation tool (High Frequency Simulation Software) by Ansoft Corporation was used to model the penetration of the EMF inside the mouse brain. This simulation showed that the waves penetrate inside the cranium of the mouse and concentrates mainly in the center of the brain.
In parallel, at SSSA, a study to understand the functioning of a nano-antenna inside the body and the influence on human tissues of radio frequency (RF) signals transmitted towards an implanted source was performed. This study was useful to fix limits for the wireless transmission power that must not be exceeded in order to avoid damage in the tissue surrounding the implanted NINIVE vector. Additionally, we studied how the EMF and the nanotube antenna can perturb cell physiology in order to induce cell permeabilization

We evaluated the feasibility of the two methodologies proposed in the NINIVE project (1)cell transfection via cells up-taking of the vectors and 2) gene transfer in the cell via electroporation) from a biomedical as well as technical point of view. Concerning the strategy n 1, the experimental results previously mentioned demonstrated that ldPEI-CNTs are able to bind DNA and such vectors have been also proved to be effective for gene transfer via natural internalization.
Our efforts were thus devoted to demonstrate that, by releasing energy to a cell with carbon nanotubes, cell permeabilization can occurs. Electropermeabilization or electroporation (EP), i.e., the application of controlled electric fields to facilitate cell permeabilization, has been known as means for introducing macromolecules, including DNA, RNA, dyes, and proteins, into cells beginning from the pioneering report of Neumann and colleagues. The electroporation mechanism is not completely understood but it is believed that pores are formed in the membrane due to the induced transmembrane voltage above a critical voltage (between 0.2 and 1V).

As first step, we develop a device to produce an enhanced electrical field at the cell membrane in contact with a carbon nanotube. Mathematical modelling and finite element modelling showed that the strong enhancement of the electrical field at the contact interface CNT/cell allows locally to overcome the transmembrane potential, making thus possible the cell permeabilization. Experimental results confirmed the theory.
As second step, we demonstrated that wireless transfection can be performed by irradiating bacteria cells with EM fields in presence of CNTs. CNTs covalently coated with low density PEI (25 kDA) were used in the experimental assays. These preliminary tests were very important to demonstrate the effect of CNTs as energy transducers: preliminary tests showed an efficiency increase of about 50% in cells incubated with DNA+CNTs in comparison with the cells incubated with DNA only and exposed at the same electro-magnetic field. 8 sample were tested (ldPEI-CNT:DNA, ldPEI:DNA, CNT and DNA, each one with and without the EM field).
As final step, we tested the antenna properties of the carbon nanotubes on mammalian cells (mouse fibroblast NIH-3T3). For these studies we selected the cytotoxic agent doxorubicin (dox) in view of its fluorescent properties as the means for investigating the effect of the EMF and MWCNTs on the uptake and intracellular distribution of this pharmaceutical. Time lapse recordings showed the application of the EMF enhance drug uptake and preferential nuclear localization and the process is strongly enhanced in presence of MWCNTs.
In conclusion, this work allowed:

- to validate our methodology
- to identify the range of working parameters (in terms of power and exposure time of the electromagnetic field)
- to test different experimental configurations of the applied electromagnetic field
- to analyse the experimental results and to point out eventual critical factors or risk elements.

The collected data leaded to in-vivo experiments on wireless transfection in mice.

The data obtained from these in-vitro and in-vivo studies confirm that MWCNTs possess antenna-like properties and when exposed to EMF in the microwave range induce non-contact electroporation of genes in-vivo. Two reporting gene were tested: GFP and Bcl-2. In mice injected with MWCNT:GFP suspension and then exposed to EMF, the expression of the ectopic DNA was confirmed in several cells of the motor cortex by direct observation using confocal microscopy. In the control (contralateral M1), GFP expression was not detected. Likewise, no expression was observed in mice injected with MWCNT:GFP but not exposed to EMF. A similar result was obtained by analyzing the expression of Bcl-2 plasmid by mmunohistological analysis of cortical expression of human Bcl-2 protein performed by using a human-selective antibody. Propidium Iodide (PI) fluorescence detected in either of the injected hemisphere was comparable, indicating that there is no loss of viable cells in neither of the two motor cortex.

Several press releases to European journals were made. The members of the Consortium have disseminated the project findings at international conferences, establishing contacts and collaborations with various groups working in the same field.

To date, the project has generated the following major publications, patent and presentations:

ISI Journals

- V. Raffa, G. Ciofani, O. Vittorio, C. Riggio, and A. Cuschieri, Physico-chemical properties affecting cellular uptake of carbon nanotubes, Nanomedicine (Future Medicine), 5:89 (2010)
- V. Raffa, G. Ciofani, O. Vittorio, V. Pensabene, and A. Cuschieri, Carbon nanotube-enhanced cell electropermeabilization, bioelectrochemistry, doi:10.1016/j.bioelechem.2009.10.006
- O. Vittorio, V. Raffa, and A. Cuschieri, Influence of purity and surface oxidation on cytotoxicity of multi-wall carbon nanotubes with human neuroblastoma cells, Nanomedicine 5:424-31. (2009)
- G. Bardi, O.Vittorio, M. Maffei, M. Costa, T. Pizzorusso, Adipocytes differentiation in the presence of Pluronic F-127 coated nanotubes, 5:378-81 (2009).
- G. Bardi, G. Ciofani, V. Raffa, M. Costa, T. Pizzorusso, Pluronic-coated carbon nanotubes do not induce degeneration of cortical neurons in vivo and in vitro, Nanomedicine: Nanotechnology, Biology, and Medicine 5, 94 (2009)

- V Raffa, O Vittorio, C Riggio and A Cuschieri, Progress in Nanotechnology for Healthcare, Minimally Invasive Therapy and Allied Technologies (Publisher ISIS Medical Media), in press
- Podesta J. E., Al-Jamal K. T., Herrero M. A., Tian, B., Ali-Boucetta H., Hegde V., Bianco, A., Prato, M., Kostarelos, K. (2009) Antitumor Activity and Prolonged Survival by Carbon-Nanotube-Mediated Therapeutic siRNA Silencing in a Human Lung Xenograft Model. Small.5:1176-1185.
- V. Raffa, G. Ciofani, S. Nitodas and T. Karachalios, D. D'Alessandro, M. Masini and A. Cuschieri *Can the properties of carbon nanotubes influence their internalization by living cells?*, Carbon, 46: 1600-10 (2008)
- G. Ciofani, V. Raffa, V. Pensabene, A. Menciassi, P. Dario, *Dispersion of multi-wall carbon nanotubes in aqueous Pluronic F127 solutions for biological applications*, Fullerenes, Nanotubes and Carbon Nanostructures, 17: 1–15, 2009
- Ciofani G., Obata Y., Sato I., Okamura Y., Raffa V., Menciassi A., Dario P., Takeda N., Takeoka S. - *Realization, characterization and functionalization of lipidic wrapped carbon nanotubes* - Journal of Nanoparticle Research, 11, 2009, 477
- L. Lacerda, S. Raffa, M. Prato, A. Bianco, K. Kostarelos, *Cell-Penetrating Carbon Nanotubes in the Delivery of Therapeutics* Nano Today 2007, 2 (6): 38-43
- M. Marchi , A. Guarda, A. Bergo, N. Landsberger, C. Kilstrup-Nielsen, G. Ratto, M. Costa, *Spatio-Temporal Dynamics and Localization of MeCP2 and Pathological Mutants in Living Cells*, Epigenetics 2007, 2 (3): 17965612

Patents

- A. Ziaei – M. Le Baillif - V. Raffa – G. Ciofani – O. Vittorio – C. Riggio – A. Cuschieri – T. Pizzorusso – M. Costa – G. Bardi – L. Gherardini – K. Kostarelos – K. T. Al-Jamal – A. Nunes – S. Nitodas – T. Karachalios, Procédé de poration de cellules comprises dans un substrat comportant l'utilisation de nanotubes comme récepteur hyperfréquences, N° of deposit: 09 05511
- V. Raffa, A. Menciassi, V. Pensabene, G. Ciofani, O. Vittorio, P. Dario, *Method and device for non-invasive in vivo electroporation mediated by carbon nanotubes*, PCT/IB2007/054754, November 22, 2007

Presentations at national and international Conferences

- "Study And Design Of Drug Delivery Systems For Cell Therapy", Gianni Ciofani, 2nd ESF Summer School Nanomedicine, Lisbon, 12-16 June 2009
- "A carbon nanotube based nanodevice for gene therapy". Raffa, G. Ciofani, O. Vittorio, T. Pizzorusso, G. Bardi, M. Costa ,K. Kostarelos, K. Al-Jamal, A. Zhiaei, S. Nitodas, T. Karachalios, M. Burghard, S. Padmanabhan and A. Cuschieri, EuroNanoForum 2009, 2-5 June, 2009, Prague, Czech Republic
- "A carbon nanotube based nanodevice for gene therapy",V. Raffa, G. Ciofani, O. Vittorio, T. Pizzorusso, G. Bardi, M. Costa ,K. Kostarelos, K. Al-Jamal, A. Zhiaei, S. Nitodas, T. Karachalios, M. Burghard, S. Padmanabhan and A. Cuschieri, Nanotech Insight Conference, Barcellona, March 29, Spain, 2009.
- "Chondroitinase ABC enhances motor skill recovery after focal ischemic injury in rat cortex", Gherardini L. & Pizzorusso T, FENS 2008, Ginevra, July 2008
- "Potential of carbon nanotubes for healthcare", A. Cuschieri, National UK EPSRC meeting (2007)
- "Nanotechnologies for Cellular Surgery", A. Cuschieri, SMIT Congress Vienna (2008)
- "Drug delivery systems", A. Cuschieri, EAES Congress, Prague (2009)
- "Nanotechnology for health", A. Cuschieri, Society of Minimally Invasive Therapy Sanai (2009)
- T. K. Karachalios and S. F. Nitodas, "Influencing Factors towards a Scalable Synthesis of Aligned Carbon Nanotubes by Chemical Vapor Deposition", ECS Trans., 25, 749 (2009)
- Nunes, A., Al-Jamal K.T., Kostarelos K. Polymer-functionalised Carbon Nanotubes for Gene Drug Delivery, Bioengineering 2009, Oxford, 24-25 September 2009
- V. Raffa, V. Pensabene, G. Ciofani, A. Menciassi, *Carbon nanotube based vectors for gene therapy*, at the International School on Advanced Material Science and Technology, VIII Course, Industrial Application of Nanotechnologies", Iesi, September 2006

- V. Pensabene, O. Vittorio, V. Raffa, A. Menciassi, P. Dario, *Investigation of CNTs interaction with fibroblast cells*, Proceedings of the 29th Annual International Conference of the IEEE EMBS Cité Internationale, Lyon, France, August 23-26, 2007: 6620-6623
- V. Raffa, V. Pensabene, G. Ciofani, A. Menciassi, P. Dario, *Gene therapy via CNTs-based artificial vectors*, VII Convegno Nazionale Istituto Nazionale di Biostrutture e Biosistemi, Rome, October 19-20, 2006.
- V. Pensabene, O. Vittorio, V. Raffa, A. Menciassi, P. Dario, Cell motion and sorting with magnetic carbon nanotubes, 6th International conference on Fine Particle Magnetism, Roma, October 9-12, 2007.
- V. Raffa, G. Ciofani, O. Vittorio, T. Pizzorusso, G. Bardi, M. Costa, K. Kostarelos, K. Al-Jamal, A. Zhiaei, S. Nitodas, M. Burghard, S. Padmanabhan and A. Cuschieri, *A carbon nanotube based nanodevice for gene therapy*, 1st Symposium on Carbon Nanotubes in Biology, Medicine & Toxicology, Montpellier, France, June 28, 2008.
- O.Vittorio, V.Raffa and A.Cuschieri, Biocompatibility of multi-wall carbon nanotubes on human neuroblastoma cell line and effects of metal impurity and surface oxidation, 1st Symposium on Carbon Nanotubes in Biology, Medicine & Toxicology, Montpellier, France, June 28, 2008.
- G. Bardi, P. Tognini, G. Ciofani, V. Raffa, M. Costa, T. Pizzorusso, *Pluronic coated carbon nanotubes do not induce degeneration of cortical neurons in vivo and in vitro*, 1st Carbon Nanotube in Biology, Medicine & Toxicology Symposium (Montpellier, France, 28th June 2008)

A NINIVE web site was established. This aims at divulging, with the necessary IPR restrictions, the main innovative aspects, which have emerged during the development of the project (*http://www.ninive-project.org)/*). The website home pages have active links to the following European organizations: http://cordis.europa.eu/ **, European Commission services,** http://www.esgct.org, **European Society Gene and Cell Therapy (ESGCT),** http://www.emea.europa.eu/, **European Medicines Agency (EMEA)Link,** http://www.carbio.eu/carbio, **CARBIO** (Multifunctional Carbon Nanotubes for Biomedical Applications), http://www.clinigene.eu/, **CLINIGENE-NoE (Network of Excellence),** http://www.nanowerk.com/nanotechnology/labs.html, A Nanotechnology Portal.

A **brochure** of the project has been uploaded on the website (http://www.ninive-project.org/Download%20Area.html): all the website visitors can download and print the leaflet for dissemination purposes. A **movie** which describes and illustrates the main concepts of NINIVE project has been commissioned and produced by an external company (Beglam SRL). It can be downloaded from the NINIVE website at the page http://www.ninive-project.org/Download%20Area.html**.** A **video** of the project activities has also been produced. This focuses on the demonstration of the NINIVE vectors as antennas for intracellular drug delivery and gene therapy and will be used as material for the next substantive publication.

Useful contacts:
a.cuscheri@sssup.it (Prof. Alfred Cuschieri)
v.raffa@sssup.it (Dr. Vittoria Raffa)

Project Logo:

Non Invasive Nanotransducer for In Vivo gene thErapy, Proposal/Contract no.: STRP 033378