PROJECT PERIODIC REPORT

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Project acronym: **NANOMMUNE**

Project title: **Comprehensive assessment of hazardous effects of engineered nanomaterials on the immune system**

Funding Scheme: **Collaborative project**

Date of latest version of Annex I against which the assessment will be made:

Periodic report: **1st** ■ **2nd** □ **3rd** □ **4th** □

Period covered: **from 08/09/01 to 09/02/28**

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[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm ; logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

NANOMMUNE Publishable summary

NANOMMUNE

The NANOMMUNE project was launched on September 1st 2008 and will run for 3 years, concluding in August 2011. The project is funded by the European Commission through the 7th Framework Programme by the funding scheme of Collaborative projects, in the area of Nanosciences, Nanotechnologies, Materials and New Production Technologies (NMP).

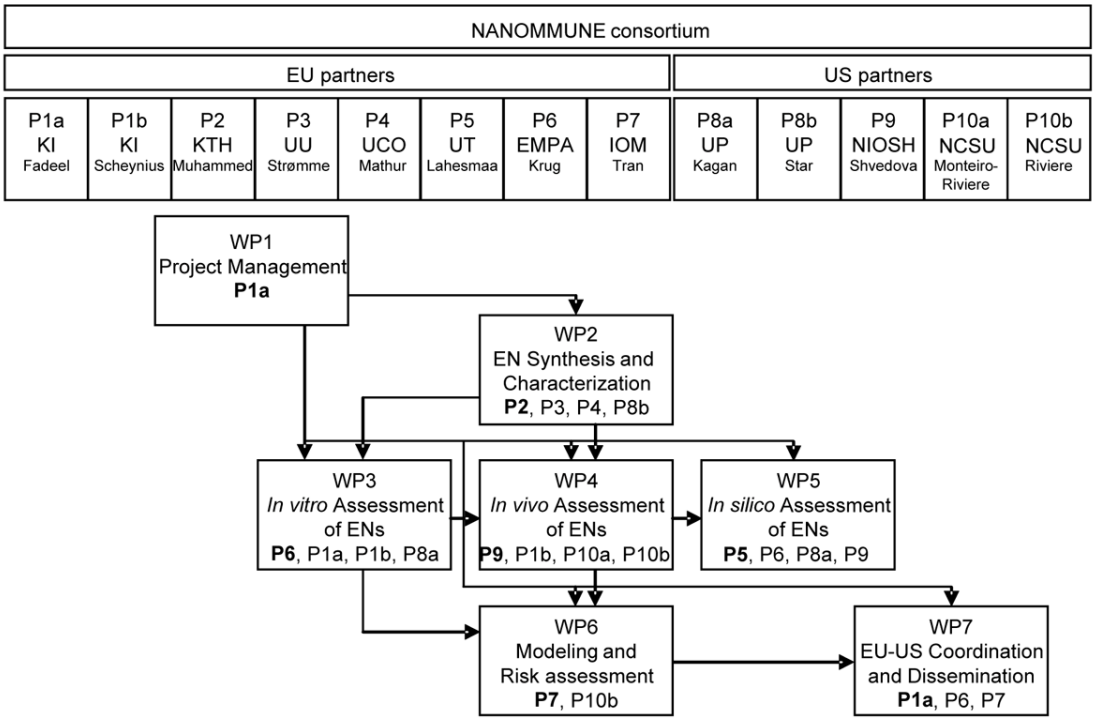
Summary description of the project objectives

Engineered nanomaterials (ENs) present tremendous opportunities for industrial growth and development, and hold great promise for the enrichment of the lives of citizens, in medicine, electronics, and numerous other areas. However, there are considerable gaps in our knowledge concerning the potential hazardous effects of ENs on human health and the environment. Our research consortium, which we have designated NANOMMUNE, is committed to filling these knowledge gaps through a comprehensive assessment of ENs, with particular focus on effects on the immune system, our primary defense system against foreign invasion.

One challenge in evaluating risk associated with the production and application of nanomaterials is the diversity and complexity of the types of materials available, and the many different routes of entry and possible sites of interaction with biological systems. Our interdisciplinary project is focused on the manufacturing and detailed physico-chemical characterization of several representative classes of nanomaterials, and the monitoring of deleterious effects of these nanomaterials on the immune system, using an array of *in vitro* and *in vivo* methodologies, as well as state-of-the-art *in silico* approaches for the assessment of genomic and oxidative lipidomic “nanotoxicity-signatures”. Our studies will also include several examples of commercial ENs that are currently on the market. Moreover, we also aim to modify specific features of various classes of ENs, in order to mitigate toxic responses to these materials.

The immune system is present throughout the body and on constant surveillance, and has the capacity to respond to invasion by pathogens and foreign particles. The core concept underpinning the current project is that the recognition versus non-recognition of ENs by immune-competent cells will determine the distribution as well as the toxic potential of these novel materials. Moreover, we will assess whether ENs interfere with key functions of the immune system *in vitro* and *in vivo*, such as macrophage engulfment of apoptotic debris and antigen-presentation or exosome production by dendritic cells to lymphocytes. Through our comprehensive approach, which combines analytical procedures from many different disciplines, we aim to establish an array of read-out systems for the determination of toxicity not only of currently existing ENs, but also for the prediction of hazardous effects

of new ENs that are being developed, thus enabling a sustainable growth of the nanotechnology-based industries.
Reinforced international cooperation and sharing of data between countries and regions is of critical importance because a reliable basis for the assessment of safety of nanomaterial-based products and technologies requires the production and implementation of standardized test materials, toxicity assays, and risk assessment strategies. To this end, the NANOMMUNE consortium strives to combine the efforts of partner institutes from several EU member states (Sweden, Finland, Germany, United Kingdom), associated countries (Switzerland), and the United States (see schematic diagram below).

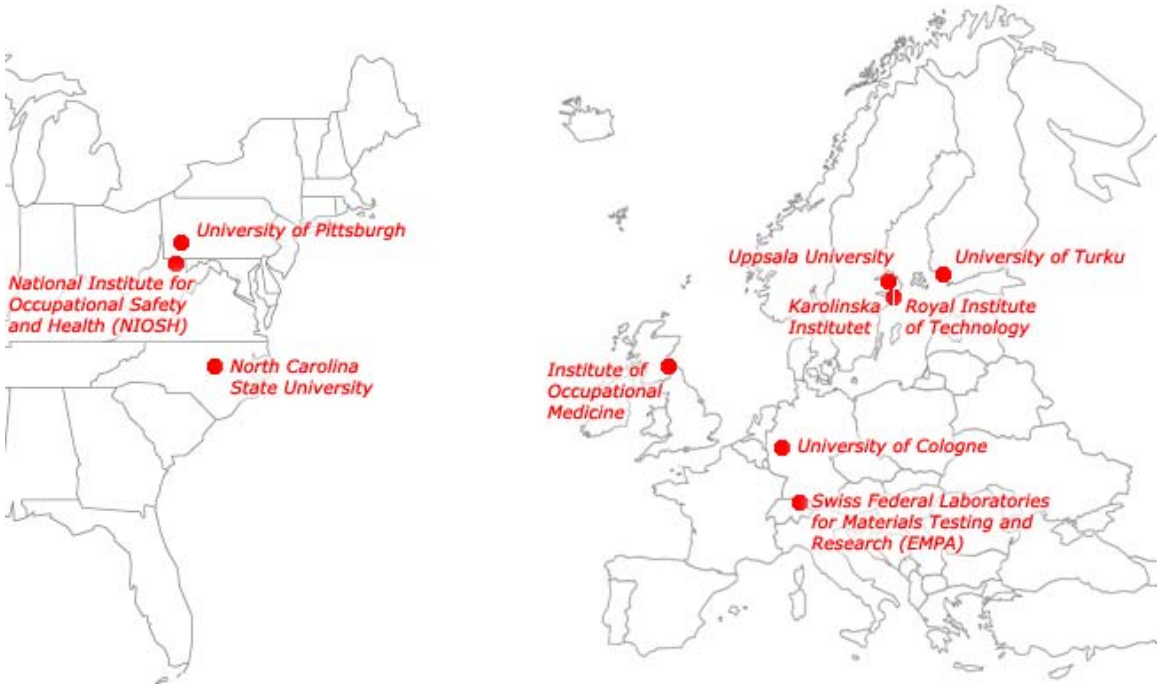


Aim

Our consortium aims to establish a panel of read-out systems for the prediction of the toxic potential of existing and emerging ENs, through a comprehensive and multidisciplinary approach based on material sciences, cell biology, toxicology, immunology, systems biology and risk assessment. Our focus is on the understanding of adverse effects of nanomaterials on the immune system.

Consortium

The NANOMMUNE consortium is coordinated by Karolinska Institutet, one of the leading medical universities in Europe. A total of 13 principal investigators belonging to 10 different partner institutes are included in the consortium. The organization of the consortium is depicted in the work package diagram above, and the geographical distribution of the partner institutes is shown below.



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3. Uppsala University (UU)- Maria Strømme
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5. University of Turku (UT)- Riitta Lahesmaa
6. Swiss Federal Laboratories for Materials Testing and Research (EMPA)- Harald Krug
7. Institute of Occupational Medicine (IOM)- Lang Tran
8. University of Pittsburgh (UP)- Valerian Kagan, Alexander Star
9. National Institute for Occupational Safety and Health (NIOSH)- Anna Shvedova
10. North Carolina State University (NCSU)- Nancy Monteiro-Riviere and Jim Riviere

Description of the work performed since the beginning of the project

The Management Office was set up at Karolinska Institutet and a project website with a public domain and a password-protected domain has been constructed. One kick-off meeting (Zurich, Switzerland, 2008) and one consortium meeting (Stockholm, Sweden, 2009) were held.

For the scientific work during the first 18 months, the main activities were concentrated to WP2 (material synthesis and characterization) and WP3 (*in vitro* assessment of ENs). To date, nanomaterials of at least 15 different systems have been studied (see project website for complete list of materials). In addition, a number of standard operating procedures (SOPs) were established for material synthesis and characterization, and for *in vitro* assessment of nanomaterials (see project website for list of SOPs). The aim is to collect all SOPs and make these available to other researchers at the end of the project (NANOMMUNE Quality Handbook). Several original articles and review articles were published, including one review in *Annual Review of Pharmacology and Toxicology*, the

leading journal in the field. For a complete list of NANOMMUNE publications, refer to the project website.

Description of the main results achieved so far

Previous studies on macrophage recognition of apoptotic cells have revealed that the exposition of the phospholipid, phosphatidylserine (PS) on the surface of apoptotic cells serves as an important recognition signal for phagocytic cells. Several partners of the NANOMMUNE consortium have now shown that coating of single-walled carbon nanotubes (SWCNT) with the "eat-me" signal, PS makes nanotubes recognizable by macrophages, including primary human monocyte-derived macrophages and dendritic cells (Konduru et al., PLoS-ONE, 2009). These studies also demonstrated that PS-coated SWCNT triggered less pro-inflammatory cytokine secretion than non-coated nanotubes. Four NANOMMUNE partners in Europe and the United States were involved in these studies. Furthermore, project partners at the University of Pittsburgh reported on horseradish peroxidase-catalyzed degradation of SWCNT (Allen et al., J. Am. Chem. Soc., 2009). These results mark a promising possibility for carbon nanotubes to be degraded in the environment.

Macrophages are the first line of defense against microorganisms and other foreign materials including particles. However, there is sparse information on the mode of uptake. We have recently reported on the interactions between mesoporous silica particles with human monocyte-derived macrophages (Witasp et al., Toxicol. Appl. Pharmacol., 2009). Mesoporous silica particles with cubic pore geometries and covalently fluorescein-grafted particles were synthesized through a novel route; these particles are endowed with very high surface areas due to their porous structure. Efficient and active internalization of mesoporous silica particles of different sizes was observed. It is noteworthy that macrophage uptake of the mesoporous silica particles was independent of serum factors. The particles did not impair cell viability or function of macrophages, including the ingestion of different classes of apoptotic or antibody-opsonized target cells. These findings are relevant to the development of mesoporous materials for drug delivery and other biomedical applications. Two NANOMMUNE partner institutes contributed to these studies.

Expected final results and their potential impact and use

The multidisciplinary approach of the current project will contribute to the elucidation of the hazardous effects of ENs on the immune system, and will enable reliable and sound assessment of the risks to human health posed by these materials. Our studies will benefit a) *citizens*, because we address issues related to human health; b) *researchers*, because we will generate new knowledge in material production, and on mechanisms of interaction of nanomaterials with biological systems; and c) *industry* (including SMEs), because we plan to incorporate our characterization protocols into a Quality Handbook, which can provide support to interested parties.

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