NANOTRUCK

Multifunctional gold nanoparticle for gene therapy

The project intends to develop an innovative kind of multifunctional gold nanoparticles loaded with fluorescent and tumoral markers, cell penetrating peptides and siRNA complementary to the proto-oncogene myc. This biofunctionalization will be tested in different biological systems, ranging from in vitro cultured human cells to in vivo animal models (primitive Hydra and complex vertebrate mouse). Expected results: development of a safe, efficient, specific, and non-pathogenic vehicle for the delivery and self-tracking of therapeutic siRNA.

Chemical synthesis

Biological assays

Partner 1 (Coordinator): Instituto de Nanociencia de Aragón Zaragoza (Spain): Prof. R. Ibarra; Dr. JM. de la Fuente
Partner 2: Centre for Cell Engineering. University of Glasgow (UK): Dr. Catherine Berry
Partner 3: Istituto di Cibernetica “E. Caianiello”, Pozzuoli (Italy): Dr. Claudia Tortiglione
Partner 4: Helmholtz Zentrum Munchen. Munchen (Germany): Dr. Furong Tian

STRATEGY

We set up effective conjugation strategies to combine, in a highly controlled way, biomolecules to the surface of AuNPs with specific functions such as: Biofunctional spacers (PEG) to increase solubility and biocompatibility and confer chemical functionality; Cell penetrating peptides: to overcome the lipophilic barrier of the cellular membranes and deliver molecules into cells; Quaternary ammonium: to introduce stable positively charged on gold nanoparticles surface; small interfering RNAs: to achieve gene silencing. siRNA complementary to a master regulator gene, the proto-oncogene c-Myc, were selected. Myc is involved in many aspects of cell physiology, and it is found deregulated in the majority of human cancers, thus is
downregulation may be exploited for lung cancer treatment.

**RESULTS**

In order to obtain a rational design of future siRNA-AuNPs, two different approaches have been used to link siRNA to the surface of the AuNPs: 1) ionic approach, where the siRNA is linked to positively charged AuNPs just by ionic interactions; 2) covalent approach, where thiolated siRNA sequences were used to allow further Au-S bonds between the NPs and the siRNA. All the AuNPs were fully characterized by different chemical and physical techniques, such as TEM, SEM, DLS, z-potential, FTIR, UV/Vis. The chemical functionalization were also characterized and quantified to provide number of PEG chain, peptides or siRNA molecules per AuNP, mainly using colorimetric methodologies.

In order to evaluate the efficiency of the 18 different prepared AuNPs, a hierarchical approach including three biological systems of increasing complexity were used: *in vitro* cultured human cells, *in vivo* invertebrate (freshwater polyp, Hydra) and *in vivo* vertebrate (mouse) models. Our synthetic methodology involved fine-tuning of multiple structural and functional moieties. Selection of the most active functionalities was assisted step by step through functional testing adopting this hierarchical strategy. Moreover, we have also characterized the dynamics and kinetics of the events occurring at the bio/non-bio interface, from the first interaction nanoparticle/cell membrane, to the intracellular trafficking and final extracellular clearance. By treating a simple water invertebrate (the cnidarian *Hydra* polyp) with functionalized gold nanoparticles, multiple inwards and outwards routes were imaged by ultrastructural analyses, including exosomes as novel undescribed carriers to shuttle the nanoparticles in and out the cells. From the 18 different AuNPs prepared along this project, only one was able to induce c-myc silencing in the three biological systems used. This AuNP obtained by the covalent approach and containing RGD and siRNA is for instance, a safe, non-pathogenic, self-tracking and universally valid nanocarrier that could be exploited for therapeutic RNAi. In order to validate this nanosystem as antitumoral gene therapy drug, the selected AuNP was used in two lung cancer xenograft mouse models resulting in successful and significant c-myc oncogene downregulation followed by tumour growth inhibition and prolonged survival of the animals.

**Added Value**

Thanks to NANOTRUCK funding five research teams, coming from very broad areas of research, collaborated together to this highly multidisciplinary project. The full consortium met each six months in one of the laboratories involved. However, it was at almost daily communication between the members of the consortium. Solid collaborations have been established, and we are actually applying for new calls to keep the collaboration.

**Outcomes and Impact**

As a summary we can conclude that NANOTRUCK project has provided an excellent tool for the preparation of multifunctional gold nanoparticles for siRNA delivery. *In vitro* and *in vivo* validations proved the efficiency of these nanodevices. Overall, NANOTRUCK has ended with 4 PhD thesis to be concluded in 2013, 23 published papers in high impact factor journals such as ACS Nano, Small, Biomaterials, and 39 communications to International Conferences.

The main results of NANOTRUCK were published on 2012 on ACS Nano, with the contribute of all five research groups:


Other relevant published results from NANOTRUCK involving CNR (Istituto di Cibernetica “E.Caianiello”)

- V.Marchesano, Y.Hernandez, W.Salvenmoser, A.Ambrosone, A.Tino, B.Hobmayer, JM de la Fuente and Claudia Tortiglione Imaging inwards and outwards trafficking of gold nanoparticles in whole animals. **ACS Nano** (accepted)
- A.Ambrosone and C.Tortiglione. Methodological approaches for nanotoxicology using Cnidarian models. **Toxicology mechanisms and methods, Special Issue on nanotoxicology (2013, in press)**

• Tortiglione C. An Ancient Model Organism to Test In Vivo Novel Functional Nanocrystals, Biomedical Engineering - From Theory to Applications, Reza Fazel-Rezai (Ed.), ISBN: 978-953-307-637-9, InTech Publisher