SCUOLA NORMALE SUPERIORE

RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – SECONDO ANNO
REPORT ON THE PHD ACTIVITY – SECOND YEAR

NOME E COGNOME NAME AND SURNAME	Giulia Giannone
DISCIPLINA PHD COURSE	PhD in Nanosciences

CORSI FREQUENTATI CON SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (WITH FINAL EXAM)	VOTAZIONE RIPORTATA MARK	NUMERO DI ORE HOURS
Fundamentals of Biophysics at the Nanoscale	25	
Seminari di Biofisica	29	45

CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	NUMERO DI ORE HOURS

ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	NUMERO DI ORE HOURS
Speaker at 5th International Health and Science Workshop - 3-4 September, Pisa	16
Tutorato per Corso di orientamento SOU Scuola Normale Superiore	4
NANO Colloquia 2019 "Melissa Santi"	1
Nanoinnovation 2020	20
Comsol day	8
CORSO DI ALTA FORMAZIONE COACH INDUSTRIA 4.0 Scuola Sant'Anna	46
IRON MAN Bad blood - treating cancer with an iron fist @Memorial Sloan Kettering Cancer Center - New York City (USA) Webinars serie on "BIOINSPIRATION AND BIOMIMETICS IN THE DESIGN OF NOVEL THERAPEUTIC APPROACHES" organized by CRS Italy Local Chapter @CRS_Italia	1

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ATTIVITÀ DI RICERCA SVOLTA (MAX. 8.000 CARATTERI)*
RESEARCH ACTIVITY (MAX. 8000 CHARACTERS)

My research project is developed within the "Translatable NanoTheranostics" group coordinated by Dr. Valerio Voliani (IIT) and relies in the optimization of novel hybrid nanoparticles (passion fruit-like nanoarchitectures, NAs) for biomedical and theranostics applications.
Remarkably, Passion fruit-like nano-architectures (NAs) are biodegradable and excretable nanoplatforms which jointly combine these characteristics with the appealing optical behaviors of noble metal nanoparticles, can offer a new alternative for theranostic applications.

My first year PhD was devoted in :
1. Study and analysis of the persistence of metals nanoparticles in the body in order to promote innovative and non-invasive treatments of oncological pathologies with the publication "**Biosafety and Biokinetics of Noble Metals: The Impact of Their Chemical Nature**" in which the chemical nature of the three most interesting noble metals for biomedical applications (gold, silver, platinum) were quantitatively correlated with their biosafety and biokinetics in zebrafish and murine models respectively to investigate fate and I different excretion paths depending on their intrinsic metallic nature.

2. New solutions for the synthesis of “ultrasmall in nano-passion-fruit like nanoparticles architecture ", in particular the use of new materials to improve the performance of the process from an economic / waste point of view and from a functional point of view in order to promote innovative treatments.

During the second year PhD my project focused in the following topics:

- Evaluation of different routes of administration of nanoparticles in murine models
- Large scale production of a new cheaper class of nanoparticles for the development of future cancer vaccines
- Biological evaluation of nanoparticles fate in cells
- Optimization of a new protocol for the synthesis of DNA-loaded nanoparticles for gene delivery and immunotherapy

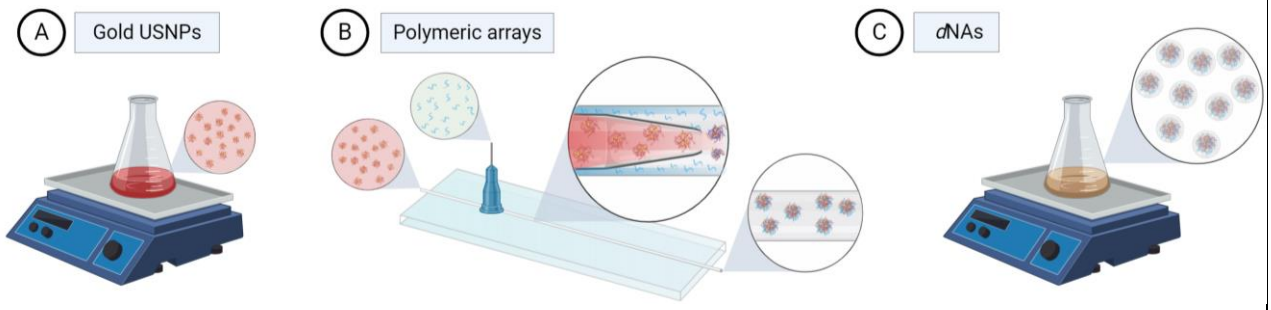
The first topic was focused on the study of the biokinetics, distribution, and clearance trends of gold ultrasmall-in-nano architectures administered through the intranasal route in murine models (with the publication "**Biokinetics and clearance of inhaled gold ultrasmall-in-nano architectures”**). Among the organism's entry portals, the respiratory tract is one of the most promising routes for non-invasive administration of therapeutics and our findings confirmed the localization of NAs in the lung parenchyma, the translocation of metal nanoparticles to secondary organs, and, following the (bio)degradation of NAs with almost complete excretion of the metal from the organism within 10 days overcoming the persistence issue. Interestingly, we have also

demonstrated that our nanoparticles were able to accumulate in the central nervous system through the olfactory neuronal pathway and escape the brain after (bio)degradation. This work paves the way for the development of systemic or local pulmonary-delivered noble metal-based treatments for oncology and infectious diseases.

Unless all the advantages that our system can hold, some practical aspects need to be taken into account while designing a nanosystem, such as ease of synthesis, reproducibility and most important the production should be cost-effective and reproducible. Accordingly, I started devoting my attention to the synthetic process optimization by changing some parameters such as materials and process parameters. In the last months, I optimized the synthesis of a new cheaper class of degradable, ultrasmall-in-nano-architectures (dragon fruit NAs, dNAs) using polyethyleneimine (PEI) as a cationic polymer instead of poly-L-Lysine (PL) without affecting either their compositions or their physiological behaviors, compared to the previous NAs. In particular, this new class ensures the preparation of high gold-loading capacity nanoparticles, a peculiar characteristic that, synergically with the interesting properties of PEI, may unlock new possible applications previously precluded to the first version of NAs while reducing the hand-made production cost by three orders of magnitude. (Published: **A Cost-Effective Approach for Non-Persistent Gold Nano-Architectures Production**).

This approach, taken together with the integration with microfluidic systems to promote the semi-automatic scaling up of production, pave the way for concrete cost-effective and large-scale manufacturing of dNAs, representing an important step toward the translation of noble metal nanotheranostics. Indeed, microfluidics offers useful capabilities: it allows the use of very small quantities of reagents, it is low cost and the resulting nanoparticles show higher monodispersity and reproducibility with respect to bulk methods.

Hence I spent two months in the laboratory of professor Hélder Santos at the University of Helsinki where I had the opportunity to learn and work with microfluidics for the scaling up of our nanoparticles production. Briefly, the handmade chip consists of a simple architecture made of two concentric capillary glasses where the reagents can mix and assemble in nanoparticle arrays.



Preliminary results confirm all the advantages of using that system: high reproducible nanoparticles with higher monodispersity, higher yield, and less waste has been produced.

In parallel to dNAs production optimization, in the last months, I have started different studies about their biological behavior and internalization in head and neck cancer cells demonstrating

their easy internalization and low toxicity profile. My experiments are devoted to the study of their pathway of endocytosis using confocal microscopy to understand their biological fate to manipulate the external surface of dNAs to increase their uptake and potential therapeutic effect.

Moreover, the use of polyethyleneimine as cationic polymer in our nanosystems has open the route for new potential treatments such as gene delivery and immunotherapy.
Indeed, thanks to the high positive charges that this polymer holds it is possible to easily bind negative nucleic acid and it is possible also to modulate their proton sponge effect that promotes the lysosomal escape in cells.
In parallel with the internalization studies with dNAs I am carrying out optimization studies for the encapsulation and release of a genetic engineering tool, CRISPR-Cas9 (a molecular tool that consists of a programmable guide RNA (gRNA) and a Cas9 nuclease can be used to edit genome) inside them. The aim is to provide a new efficient non-viral vector for gene editing and to compare the transfection efficiency with commercial reagents.

In the next months, I will finalize the internalization studies and plasmid-encapsulation optimizations with correlated cell studies, and starting from January 2021, I will spend 7 months in Professor Hélder Santos laboratory to boost dNAs translation to patients, finalizing the microfluidics optimization.
In parallel to this, studies concerning the application of nanoparticles in immunotherapy as potential nanocarriers for the development of cancer nanovaccine and gene therapy will be carried out.
The present project aims to investigate the in vitro immunological profile of dNAs,
In particular,

- Evaluate the cytocompatibility of the NPs in immune cells.
- Quantify their uptake in immune cells.
- Analyze their immunostimulatory potential by varying the core composition (metal nanoparticles, polymers)
- Analyze the feasibility of a combined immuno-gene therapy treatment

*se si intende sottoporre una relazione di ricerca più estesa, utilizzare il campo per una descrizione sintetica e allegare il documento in formato .pdf
If you are going to submit a longer report, please fill the box with a synthetic abstract and attach a document in pdf format

EVENTUALI PUBBLICAZIONI
PUBLICATIONS (IF AVAILABLE)

Giannone G., Santi M., Ermini M.L., Cassano D.,Voliani V., A Cost-Effective Approach for Non-Persistent Gold Nano-Architectures Production.Nanomaterials, 2020, 10(8):1600

Mapanao A. K.*, **Giannone G.*,** Summa M.*, Ermini M. L., Zamborlin A., Santi M., Cassano D., Bertorelli R. and Voliani V., Biokinetics and clearance of inhaled gold ultrasmall-in-nano architectures. Nanoscale Adv., 2020,2, 3815-3820

Synergic antimicrobial effect of non-persistent silver nano-architectures and chlorhexidine for infected wound healing (peer review)

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Bioartificial Sponges for Auricular Cartilage Engineering May 2020
(DOI: 10.1007/978-3-030-47705-9_17) In book: Advances in Bionanomaterials II

NOME DEL RELATORE THESIS ADVISOR
Dr.Valerio Voliani

DATA DATE	17/10/2020	FIRMA SIGNATURE