PhD Course Converging in Technologies for Biomolecular Systems (TeCSBi) XXXVI cycle, a.y. 2020/2021

n.4 scholarships funded by Department

1) Project Supervisor: Prof. Paride Mantecca

Project Title: Adverse outcome pathways (AOP)-oriented toxicology in 2D and 3D in vitro systems for implementing the safety-by-design of new nanomaterials

Possible support for Phd students w/o a University fellowship:

Introduction

In the nano(bio)technology field, the Safe-by-Design concept (SbD) incorporates safety of nano-enabled product (NEP) at the design stage of the production process. SbD reverses the paradigm of downstream risk analysis and management and pursues the production of less hazardous nano-products affording reduced exposure and hazard, mediated by the release of nanomaterials (NMs) during the life-cycle. Starting from the knowledge of the structure-reactivity relationship is possible to predict the biological hazard of the new NMs and re-design the final products, once the cellular and molecular mechanisms of toxicity have been adequately elucidated.

The research activities of this PhD will be carried out in the framework of the H2020 project ASINA (Anticipating Safety Issues at the Design Stage of NAno Product Development).

Objectives

The main objectives of the project is to identify the hazard toward human health during the production and use of antimicrobial, antibiofilm and depolluting coating technologies with metal-based NMs and of nanostructured capsules delivering active phases in cosmetics. The results will

allow the identification of hazard design criteria and will be conducted in parallel to the release, fate and exposure studies, also linking the systems to the NM physico-chemical (p-chem) properties.

The hazard design criteria will be based on mechanistic toxicity data. The extrapolated results will identify the safety profiles of the new products and will feed the risk assessment process, in compliance with regulatory frameworks.

For this applicative purpose it is mandatory to develop sensitive and predictive biological systems and to investigate the molecular pathways evoked by the bio-interactions with new NMs and NEPs and the events leading to the cellular adverse outcomes.

Thus, within this project, 2D and 3D in vitro systems representative of the human skin and lung barriers will be developed and used to investigate the effects of new NMs, basing on identified adverse outcome pathways (AOPs).

Methods

In vitro testing/modelling of the hazardous properties of different metal-based NPs (including polymer-surface functionalized and hybrid NPs) and nanocapsules will be performed to detect the relationships between the p-chem properties and toxicity outcomes. In parallel the modality of nano-bio-interactions and the molecular modes of action will be investigated.

2D cell models representative of different target tissues, including epithelial cells (human lung alveolar cells, keratinocytes), fibroblasts, immune cells and advanced 3D in vitro models (Alveolar-Blood Barrier, ABB and reconstructed skin) will be used for screening cytotoxic, genotoxic and pro-inflammatory activity of the nanoparticles (NPs) specifically synthesized for the purposes of the ASINA project. The NMs and target cells/tissues will be selected according to the NPs environmental fate and exposure scenarios predicted.

When possible, standard OECD or ISO tests will be adopted to match the regulatory frameworks. At the same time, the same biological models will be used to investigate AOPs and relevant biomarkers of effects for implementing the hazard assessment and the SbD strategy.

The following toxicological approaches should be involved.

1-Inhalation toxicity. Human lung cell mono- and co-culture, as well as 3D models of the alveolar blood barrier, will be exposed in both submerged and/or air liquid interface (ALI) conditions, for direct exposure to the nano-aerosol.
2-Skin toxicity. Skin toxicity will be performed using both 2D monocultures of keratinocytes and/or fibroblasts by standard cytotoxicity tests and 3D in vitro reconstructed human skin models (e.g. EpiDerm, MatTek). The latter will be investigated by standard in vitro Corrosion Skin test and Irritation Skin test, exposed to NP suspensions and extracts from the coated materials.
Standard cytotoxicity assay, like the MTT, Alamar Blue, Colony Forming efficiency (CFE) assay and Neutral Red Uptake (NRU) will be performed after acute and sub-chronic cell exposure. In parallel, the production/release of pro-inflammatory cytokines and the genotoxic effects will be evaluated by different techniques, including WB, ELISA and cytofluorimetric techniques. Gene and protein arrays and other molecular biology techniques will be used in parallel for the identification of AOPs. Particular attention will be payed to the characterization of NP-cell interactions by mean of different microscopy techniques, including confocal and hyperspectral microscopy and the powerful Correlative Light-Electron Microscopy (CLEM) techniques.

The PhD student will work in labs specialized in nanotoxicology and nanosafety studies and will share experience with a multidisciplinary international partnership. In addition to the training in basic and advanced toxicity studies, the student will gain experience in the regulatory aspects, thanks to the collaboration with companies, and will improve the basic knowledge on the mechanistic toxicity aspects of the nano-bio-technology field.

2) Project Supervisor: Dott. Laura Russo

Project Title: Design of ECM mimetics and their application in 3D Bioprinted tissue constructs

Possible support for PhD students w/o a University fellowship:

The PhD project will train the fellow and develop a multidisciplinary research in the field of advanced 3D Extracellular Matrix (ECM) mimetics and 3D bioprinted tissue models. The project will

develop synthetic bio-inks based on FDA approved biomaterials of natural and synthetic origin, to generate 3D-scaffolds with modular morphological, chemical and biochemical properties. The strategies will require crosslinking of the starting biopolymers and their functionalization with signaling molecules in order to generate an ECM mimetics able to “dialog” with the cells and induce their fate. The project plans to exploit the bioinks to generate tailor-made, cell containing, synthetic tissue model by 3D bioprinting.

The project has a strong translational character, at it will strongly contribute to: (i) the development of new biomaterials for tissue regeneration and in vitro culture models; ii) a better understanding of the role of the ECM properties in the onset of pathologies and the validation new synthetic matrices employable also in cancer-related models; iii) the development tools based on personalized tissue models for animal-free drug testing and screening.

3) Project Supervisor: Dott. Gianni Frascotti

Project Title: Development of vault-based nanovectors for the targeted delivery of therapeutic molecules to cancer cell lines
Possible support for Phd students w/o a University fellowship: no

1. Background

Genetically engineered microorganisms are commonly used in heterologous proteins production as they can be grown at high cellular density in shake flasks and bioreactors. In this respect, the methylotrophic yeast *Pichia pastoris* is an ideal host for the expression and production of recombinant complex eukaryotic proteins, enabling their production at low costs and in large amounts, also endowed with most of eukaryotic post-translational modifications [1].

The present research project will develop nanovectors based on engineered variants of the vault nanoparticle, which will be produced in *P. pastoris*. Vault particles consist of 78 copies of the 97 kDa major vault protein (MVP), the 193 kDa vault poly(ADP-ribose) polymerase, the 290 kDa telomerase-associated protein-1 (TEP1) and one or more small untranslated RNAs [2,3]. The size of vault particles is 72.5 × 41 × 41 nm. The MVP generates a barrel-like, natural “nanocapsule” [2]

that holds a tremendous potential as a tool for drug/gene delivery. Actually, it can encapsulate drugs, proteins or nucleic acids and can be targeted to specific cell surface receptors, provided MVP is suitably modified with targeting agents.

2. Rationale

To develop nanovectors capable of delivering cytotoxic molecules to selected cancer cell lines, we will employ different variants of the vault nanoparticle, and will load it with a preselected repertoire of cytotoxic proteins or siRNAs. Vault will be also bound to specific antibodies, which will ensure a targeted delivery.

This rationale relies upon the availability of the following technological resources:

1) The vault variants of interest, produced in the industrial microbiology laboratory of the proponent using *P. pastoris* as the expression system, and purified as previously reported [4].

2) Vault derivatization with antibodies capable of selectively targeting cancer cell lines. This will be accomplished by chemical conjugation, as we already did in the case of trastuzumab (Tz), a monoclonal antibody recognizing the HER2 receptor, overexpressed in some breast cancer cell lines. However, antibodies can be also directly bound by the MVP-protein A fusion protein we have already produced.

3) Loading the vault nanoparticle with cytotoxic proteins bound to the INT domain, either chemically or genetically. INT binds specifically to MVP in vault's interior [2].

4) Loading the vault nanoparticle with siRNAs, which inhibit tumor proliferation via gene silencing. For this purpose, RNAs will be chemically linked to the INT domain.

3. Experimental design

1) Production in *P. pastoris* of the following MVP variants: a) wild type; b) the fusion protein CP-MVP, with the CP domain (a Cys-rich peptide that stabilizes MVP structure) at the N-terminus [5]; c) the fusion protein pVI-MVP with pVI at *the* N-terminus, a peptide that allows endosomal escape [6]; d) MVP in fusion at the C-terminus with protein A that binds IgGs.

We have already cloned the gene encoding the wild-type MVP in *P. pastoris* pGAPZA vector. We will isolate MVP-producing clones and search for the best-expressing ones.

Different cultivation conditions will be explored by modulating, in particular, temperature and aeration. If required, the optimized process will be scaled up in a laboratory bioreactor.

2) Purification of vault variants using our simplified procedure [7].

3) Production of vaults conjugated with antibodies capable of aiming at defined cancer cell lines. Antibodies will be linked either chemically or using the vault variant in fusion with protein A at MVP C-terminus.

Besides Tz, we will also test Cetuximab, used in the treatment of some tumors, due to its well-known capability of targeting to the epidermal growth factor receptor (EGFR).

A substantial part of the experimentation will be carried out by Prof. Flagiello's laboratory at the J. Monod Institute (Université de Paris). To target specific siRNAs to cancer cells, they will use some humanized monoclonal antibodies, directed to the receptor PD-1, specifically overexpressed in human melanoma and coupled to vault nanoparticles. These vault variants will specifically deliver some siRNAs capable of inhibiting tumor metastasis.

4) Cytotoxic cargo molecules. a) Cytotoxic proteins. We will use the vault nanoparticle to deliver either onconase or cytochrome c. Onconase is an RNase from amphibian embryos, whose cytotoxic activity and antitumor properties are well described. Conversely, the proapoptotic properties of cytochrome c are well known since over twenty years. Either protein will be chemically linked to the INT domain, or fused genetically with it. b) We will exploit the INT domain as a siRNA carrier via chemical conjugation. We will use RNA variants carrying a thiol group, to link them to GFP via a disulfide. As regards melanoma cell lines, Prof. Flagiello's team recently reported on the discovery of *LADON*, an lncRNA, and on its pro-metastatic role in melanoma, so we will load the nanoparticle with siRNAs specific to LADON. As two siRNAs cause a significant decrease in LADON expression in the A375 melanoma cell line [8], we will use them, either in isolation or conveyed by the nanoparticles.

Based on the same strategy, we will target other cancer cell lines, primarily breast cancer, using GFP-siRNAs carried by vaults.

The effects of the different nanossemblies on cancer cells will be monitored by standard methods, including standard cell viability assays, metastasis assays [8], FACS analysis, confocal microscopy. In view of these analyses, antibodies and/or vaults will be derivatized with suitable fluorophores.

References

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4) **Project Supervisor: Dott. Luca Campone**

Project Title: Foodomics approach to evaluate health benefit and toxicity of foods and nutraceutical" products
Possible support for Phd students w/o a University fellowship: Fondi Chronos

Background

Noncommunicable diseases (NCDs) also known as chronic diseases, represent about 71% of all globally deaths. Cardiovascular and cancer are considered the main causes of all premature NCDs deaths [1]. Noncommunicable diseases are the result of a combination of genetic, physiological, environmental and behaviors factors. These factors could be divided into factors that reduce NCDs incidence and mortality and factors that increase NCDs incidence as bad lifestyle and ingestion of toxic compounds, and diets play a crucial role into intake of both these substances. Food is a variable combination of nutrients, and substances with no nutritional relevance, namely

nutraceuticals, with effects on human health [2]. Nutraceuticals products included pure compound from food, dietary supplement, processed food and also herbal products [3].

Over the past few decades, the use of nutraceuticals, as dietary supplements, have increased exponentially also due to the huge quantity of internet information that magnify the advantageous properties of these products, although the safety or effectiveness have not been scientifically proven. Many studies regarding the health benefit of nutraceutical compounds are often controversial: on one side some reports suggested beneficial effects of bioactive compounds, such as epigallocatechin gallate [4], resveratrol [5] and flavonoids [6] in the prevention or treatment of several disease including cancers. On the other hands the consumption of many foods and nutraceuticals are linked to cancer development and other toxic effects. Examples of compounds found naturally that have potential carcinogenic effects are capsaicin [7], phytoestrogens [8], pyrrolizidine alkaloids [9-10]. Moreover, unexpected compounds, such as environmental contaminants or natural toxins can occur in foods and nutraceuticals, bringing about the potential health risk [11]. For these reasons the analysis aimed to discover the chemical composition of foods and nutraceutical products become a topic of enormous interest in cure and prevention of NCDs.

Rationale

This project is devoted to assessing the role of diet and nutraceuticals to prevent or promote NCDs and will focus on the development of protocols aimed to study foods and/or nutraceuticals metabolome, under two different but related point of view

i) discover health benefits of antioxidant and anti-inflammatory compounds that may reduce the risk of cancer and other NCDs

ii) study the role of carcinogens or tumor promoters that could occurs in foods and nutraceutical products.

At this purpose Metabolomics may be used as a valuable tool providing important information that links foods or nutraceuticals metabolites with human health. Metabolomics approaches are typically classified as either non-targeted or targeted [13]. Non-targeted analysis refers to a procedure in which the maximum coverage of metabolites can be simultaneously detected from complex biological matrix, whereas targeted metabolomics analysis focuses on the determination of

metabolites of a particular class or metabolic pathway. Since the metabolome consists of numerous small molecules (<1000 DA) with chemical complexity and concentration diversity, analytical technologies with high specificity and powerful quantitative and qualitative capabilities are required. Mass spectrometry (MS) will be preferred over the other techniques, due to its high-resolution specificity, and super-sensitivity enabling comprehensive quantitative and qualitative measurement of large-scale small-molecular metabolites in complex biological samples [13]. The potential of MS-based metabolomics allows to cover as many as possible groups of metabolites or many metabolites of a specific group, from every aspect as authenticity, functionality, quality and safety issues. Metabolomics procedures, including sampling, sample preparation, instrumental separation, data analysis, identification of potential candidates and biological interpretation, are often performed sequentially to complete the whole metabolomics study.

Experimental plan

The project will be divided in 2 work-packages:

WP1. Untargeted analysis for the screening and selection of wide bioactive extracts able to prevent the insurgence or progression of NCDs.

WP2. Targeted analysis for the rapid identification of toxic compounds able to promote the insurgence or progression of NCDs.

The first step will be focus on matrix selection for foodomics analysis. This screening will be carried out on both literature data and on preliminary results of in vitro test. In order to avoids bias that could compromise the whole results of metabolomics analysis, extraction methods as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) will be used, and instrumental parameters will be careful optimized by chemometric analysis (experimental design).

The obtained metabolome will be analyzed by using MS-based analytical methodologies for the detection and characterization of metabolites. These MS-based analytical methodologies generate a large amount of data, thus dedicated software to filtering, align, and process all data will be used. The results generated by this software will be processed by bioinformatic statistical data analysis as principal component analysis (PCA) and partial least squares analysis (PLS). Finally, in

order to obtain biological information from the data the identification of metabolite peaks will be performed. To identify potential candidate accurate mass of compounds will be first searched in the databases and if no database will be able to identify the metabolites more data, including accurate mass, MS/MS, or NMR spectra, will acquired for compounds elucidation. The metabolomic procedure will hopefully ends with transformation of the metabolic information obtained, into a biological interpretation, which is performed by key node analysis, correlation analysis, metabolic pathway analysis or mathematical modeling of metabolic networks.

References

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High level training apprenticeship contracts

n. 2 contracts with Istituto di Ricerche Farmacologiche Mario Negri IRCCS

1) Project Supervisor: Dott. Paolo Bigini

Project Title: Role of energetic metabolism in ovarian cancer progression and response to therapy. (The research will be carried out at Dipartimento di Oncologia- Istituto Mario Negri)
Possible support for Phd students w/o a University fellowship:

Metabolic re-programming is one of the hallmarks of tumors and recent evidence suggests that it may help determine the response and / or resistance to therapies.
The objectives of the thesis project are:
i) the characterization of the energy metabolism of ovarian tumors and the study of the influence of tumor metabolism on the host response (with particular regard to the immune response9;
ii) the identification of metabolic changes induced by different treatments (chemotherapy, angiogenesis inhibitors, PARP inhibitors) and their role in resistance to therapies;
iii) the optimization of therapies with metabolism modulators in single therapy or in combination with drugs in use, through preclinical studies on animal models of ovarian cancer.
The research project is based on the use of a biobank of xenon tumors (OC-PDX) derived from patients with ovarian cancer and stabilized in the Laboratory of Tumor Metastasis Therapy and of syngeneic mouse models of ovarian cancer. Mass spectrometry techniques will be used for the evaluation of metabolic activity in vitro and in vivo, while the identification of the various immune components will be carried out through the use of flow cytometry.

Salary for apprendice: € 15000 gross.

2) Project Supervisor: Dott. Paolo Bigini

Project Title: Role of physico-chemical features of nanoparticles on their tropism toward the target in murine models of liver pathology. (The research will be carried out at Dipartimento di Oncologia- Istituto Mario Negri)
Possible support for Phd students w/o a University fellowship:

The main aim of this project is to understand the influence of physico-chemical features of nanoparticles on their potential effect. For this purpose, a wide range of nanovectors will be tested in terms of biodistribution, organ accumulation, targeting and potential toxicity. In the second part, drugs will be linked to the most efficient nanocarriers to carry out pharmacokinetic and pharmacodynamics studies. Last, the therapeutic efficiency of the selected nanodrugs will be tested in murine models of human disorders. The thesis project will include both in vitro an in vivo studies.

The tight interaction with both chemists and medical doctors will allow to the student to follow the whole pipeline related to a project of drug development.

Salary for apprendice: € 15000 gross

Posizioni con Percorso Executive

n.1 posizione riservata a dipendenti del Cotonificio Albini SpA e vincolata al progetto di ricerca:
Gli enzimi nel settore tessile: Le potenzialità dei sistemi enzimatici a sostegno della circolarità

Studio delle potenzialità dei processi di bio-trasformazione nel settore tessile a supporto dello sviluppo economico circolare.

Profilo
Dottore di Ricerca con Esperienza nel campo delle trasformazioni di sottoprodotti di filiera industriale in una logica di bio-raffineria.

Progetto

Il progetto di Dottorato ha lo scopo di studiare, sia nella teoria che nella pratica, le potenzialità dei sistemi enzimatici e dei processi biotecnologici, con una particolare attenzione per quelli basati su microrganismi, nel settore tessile.
La ricerca si propone di studiare la potenzialità di enzimi naturali o ingegnerizzati nella trasformazione di sottoprodotti della filiera riducendo le fibre ai loro costituenti, in particolare la cellulosa a monomeri di glucosio.
Durante il progetto verranno studiati anche i possibili impieghi degli enzimi sia all'interno dei processi produttivi tessili che a favore del recupero della polpa di cellulosa, cercando di costituire una sinergica collaborazione tra Università e Azienda in un'ottica di circolarità.

Ore di formazione esterna (in Università)
180 (nei 3 anni) Parte delle ore (fino al 40%, ovvero 72 ore) possono essere sostituite da attività formative ad hoc per l'acquisizione di competenze specifiche, da concordare con il dottorando e con il tutor aziendale in base alle esigenze del progetto.

Esami obbligatori
Acquisizione di almeno 14 CFU nel corso dei 3 anni, per lo sviluppo di competenze complementari.