Nutraceuticals and Delivery Lab

Proposals for MSc Research Projects 2013/14

TITLE: Development of a "green process" for the isolation of natural functional extracts with anti-cancer activity - Application of high-pressure technology

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This Master Plan is part of a major project whose main goal is to develop adequate clean and mild methodologies for the isolation or fractionation of terpenes-containing extracts (in particular perillyl alcohol (POH)-rich extracts from cherries, citrus, mint and Lavender), alternatively to conventional harmful solvents extraction. Either specific processes or integrated methodologies are being explored for tuning the chemical compositions and targeted characteristics of end-products. SFE (Supercritical Fluid Extraction and PLE (Pressurized Liquid Extraction) will be explored as reliable tools to extract POH from different natural sources, namely cherries, citrus, mint and lavender.

These technologies are safe and environmentally friendly (all the solvents employed are non toxic) and thus the final product is regarded as natural, being allowed for food and pharmaceutical applications.
The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling high pressure apparatus) and in diverse analytical techniques for the bioactive-rich extracts phytochemical characterization.
The student will perform diverse analytical techniques for the bioactives quantification and also evaluate the efficacy of the extracts as chemotherapeutic candidates through in vitro cell bioassays.

TITLE: Preparation of functional microsystems with application in cellular expansion and differentiation - Application of supercritical fluid technology

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Recently, pharmacologically active microcarriers (PAM) has been developed to overcome certain problems encountered in cell therapy, particularly cell survival, lack of cell differentiation and integration in the host tissue. Clean processes such as supercritical fluid technology has been applied as alternative to conventional precipitation methods to produce controlled-release microparticulate systems.

The aim of this project is to exploit and optimize a (SCF)-based precipitation technology (as alternative to conventional processes) to produce pharmacologically active microcarriers (Microsystems) for a sustained and controlled-release of a growth-factor that promotes cellular expansion and differentiation. The materials to be explored as carriers are polymers (or mixtures of) such as poly(D,L-lactic-coglycolic acid), chitosan, polyethylene glycol (PEG).

These (SCF)-based precipitation technologies are safe and environmentally friendly (all the solvents employed are non toxic) and thus the final product is regarded as natural, being allowed for pharmaceutical applications.

The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling supercritical fluid precipitation apparatus) namely in supercritical fluid precipitation techniques.

The student will learn how to perform physico-chemical characterization of the Microsystems prepared that involves diverse methods of solid-state analysis (e.g. morphology, structure organization, particle size distribution) and evaluation of the process efficiency (through growth-factor release profile and functionalization efficiency).

TITLE: Particle engineering for the optimization of Pharmacologically active microcarriers

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Pharmacologically active microcarriers (PAMs) must be biodegradable and non-cytotoxic microspheres, with a sustained and controlled-release of a therapeutic protein such as a growth factor, with application in cellular expansion and differentiation.

The aims of this project is to test diverse pharmacologically active microcarriers, produced with different biomaterials combinations and functionalized with specific growth factors (or other proteins) and evaluate their efficacy on the in vitro cellular expansion and/or differentiation of different stem cell types.

PAM's will be produced for a sustained and controlled-release of growth-factors. The materials used as carriers are biopolymers and natural polysaccharides (or mixtures of) such as poly(D,L-lactic-coglycolic acid), polyethylene glycol (PEG); chitosan and alginate.

The student enrolling in this project will have opportunity to acquire expertise in conventional precipitation techniques and physico-chemical characterization methodologies to prepare and characterize PAM's, respectively. Moreover, the student will learn cell manipulation techniques to evaluate the PAMs cytotoxicity and their efficacy in supporting stem cell expansion and/or differentiation into specific cell lineages (e.g cardiomyocytes, neurons).

TITLE: Evaluation of neurotoxicity of solid lipid nanoparticles: uptake and cellular response using an human neuroblastoma cell line.

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Solid lipid nanoparticles (SLNs) have been developed as promising targeted drug delivery systems to the brain and have been proposed as alternative carriers to polymeric nanoparticles to overcome some of their common problems. Lipid nanoparticles are generally made up of biocompatible lipids and natural surfactants and due to their unique sizes and properties, SLNs offer great promise to develop new therapeutic approaches, addressing challenges of drug loading and drug targeting. However, toxicity of these new formulations has not been investigated thus far.

The aim of this proposal is to evaluate the potential neuro-nanotoxicity of SLNs using a human cell line as neurons model. The nanoparticulate systems – cell interactions will be studied through the evaluation of their cellular uptake and by the impact of SLN specific properties (e.g. particle size, surface structure, morphology and hydrophilic-lipophylic balance) on biological processes such as oxidative stress response, cell death and expression of inflammatory mediators.

The student enrolling in this project will have opportunity to learn animal cells manipulation techniques and biochemical methodologies as ELISA, western blot analysis and Flow Cytometry.
Moreover, the student will have opportunity to acquire expertise on i) preparation of SLNs using conventional and non-conventional precipitation techniques (e.g. spray chilling and particles from gas saturated solutions) and ii) physic-chemical characterization of the nanoparticulate systems that involves diverse methods of solid-state analysis (e.g. morphology, structure organization, particle size distribution).
This proposal is part of a major project that intends to develop innovative targeted drug delivery systems.

TITLE: Evaluation of immunomodulation properties of b-glucans on human enterocytes

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Glucans are glucose polymers, classified according to their interchain linkage as being either α- or β-linked. β-glucans are a heterogeneous group of nonstarch polysaccharides, consisting of D-glucose monomers linked by β-glycosidic bonds.

β-glucans can vary in terms of frequency, length and degree of branching, degree of branching, molecular weight (from 102 to 106 daltons), polymer charge, and/or solution conformation (random coil or triple or single helix) as well as solubility.
All these physicochemical characteristics and the β-glucans macromolecular structure depend on both the source and method of isolation/extraction of β-glucans and play a role in the shaping of β-glucan-associated biological activities.

The immunological properties of β-glucans varies with properties such as the molecular mass, solution conformation, backbone structure, degree of branching as well as the cell type that is targeted.

In this work, we aimed to evaluate immunomodulating properties of β-glucans, produced from waxy varieties of barley using different recovery processes, on inflamed intestinal epithelial cells. The influence of the recovery process applied as well as the physicochemical properties of the several β-glucans ended products (molecular weight, degree of purity, co-extracted products, …) will be studied.
The student enrolling in this project will have opportunity to learn animal cells manipulation techniques and biochemical methodologies as ELISA, western blot analysis and Flow Cytometry.

TITLE: Nutraceuticals from *Brassicaceae*: Solubility measurements of bioactive compounds from crucifeous vegetables in supercritical carbon dioxide.

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Cruciferous vegetables (Brassicaceae family) are recognized as an important source of biologically active compounds with a large spectrum of biological actions. In fact, several studies linked the presence of glucosinolates and its hydrolysis products, isothiocyanates (ITCs) with the observed health-promoting effects, particularly, chemoprotective activity. Particularly, ITCs are pointed as the phytochemicals responsible for the beneficial properties of cruciferous but their intake in daily diet may not be sufficient to achieve those claimed effects mainly due to heat treatment during cooking.

The work to be developed is part of a major project whose main goal is to design, optimize and develop high pressure methodologies (such as supercritical fluid extraction or pressurized liquid extraction with CO2) to obtain high value extracts with health-promoting activities from cruciferous vegetables by-products.
The work planned for this proposal is essential for the design, development and optimization of the high-pressure extraction procedures since the results obtained will strongly influence the selection of processing conditions to be adopted. The student will perform solubilities measurements of targeted bioactive molecules present in cruciferous vegetables in supercritical CO2 [x bioactive compound + (1-x) CO2] and evaluate the impact of the use of diverse co-solvents.
The student enrolling in this project will have opportunity to acquire expertise in high-pressure technology (handling high pressure apparatus), namely supercritical fluid technology and perform diverse analytical techniques for the bioactives quantification.

TITLE: Characterization of antioxidant capacity of food and beverages - Evaluation and establishment of structure- activity relationships.

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Polyphenols are the most abundant antioxidants in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee, wine and fruit juices.
Total phenolic content (TPC) determined using Folin-Ciocalteau method and other fluorimetric assays (like ORAC e HORAC) have been used to evaluate and compare phenolic composition and antioxidant capacity of foods, related products or extracts. However it is known that sample matrix can interfere in this measurements, as the chemical reaction occurring is not specific from phenolic compounds and ascorbic acid, proteins, sugars may interfere in the determination. Also phenolic compounds from different families may not give the same kind of response.
The aim of this work will be:
(i) selection of a standard mixture representative of samples (food and related samples) understudy and simulation of the contribution of different interfering compounds. In parallel results will be controlled by LC-DAD;
(ii) to evaluate and conclude about the response of different standards from different families of phenolic compounds to different antioxidant capacity assays;
(iii) compare results obtained with Folin Ciocalteau reagent and detection at 280 nm using LC-DAD for the different samples.

TITLE: Evaluation of cardioprotective effect of natural ingredients

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The antioxidant effect of many compounds present in natural sources has received an increased interest in food and pharmaceutical industries. Today many foods and supplements claiming health benefits are available in the market. For the development of these functional products, more robust and physiological relevant cell based assays are required prior to the antioxidant research in human clinical trials.

The main aim of this project is to develop 3D cell-based bioassays for the evaluation of the antioxidant properties and cardioprotective effect of natural ingredients. This will be achieved by using induced pluripotent stem cells (iPSCs) from human and murine origin as a renewal source of functional cardiomyocytes (CMs). Cell culture will be performed using 3D cell aggregates in environmentally controlled stirred tank bioreactors operating at physiological conditions. After differentiation, 3D aggregates of CMs will be transferred from

stirred tank bioreactors to static non-adherent culture systems (well plates) and oxidative stress will be promoted using different chemical inductors (e.g. H2O2, doxorubicin). Oxidative damage in CMs will be evaluated by measuring cell viability (measured by alamarBlue assay, MTT, etc) and functionality at the level of oxidative-reduction status (e.g. ROS production) and oxidative damage (proteins, lipids and DNA oxidation). Additionally, morphological characterization and functionality of CMs will be assessed using immunofluorescence confocal microscopy tools and electrophysiology tests. After the implementation of an oxidative stress model for 3D cardiac cells, the cardioprotective effect of standard antioxidant compounds and natural ingredients derived from several matrices (apples, grapes, cherries, cactus pears, etc) will be evaluated. The antioxidant effect of the natural extracts will be correlated with the composition of samples aiming at identifying the most promising cardioprotective compounds.