# MSc Project

**TITLE**

Dynamics of circulating coelomocytes populations during star-fish regeneration

**BACKGROUND**

The potential for regeneration has its maximum expression in echinoderms. Preliminary studies of regeneration in echinoderms were based on the determination of growth rates and on the morphological, histological and celular basis of this phenomenon. More recently, some advances have been made in the characterization of the molecular mechanisms involved in the regeneration process of tissues and organs. Studies that have been developed in our laboratory focus on a star-fish species common in Portuguese coastal areas and with high regeneration capability, the Marthasterias glacialis. Coelomic fluid, the main intra-tissue communicating medium in echinoderms, contains different types of cells, generally called coelomocytes, which are believed to participate in several functions such as, nutrient storage, gas exchange, production of connective tissue components, immune defense and tissue regeneration upon natural amputation. Although there is a generally accepted morphological identification of five types of coelomocytes there is no uniform criteria until now on their classification, as well as a correlation between morphology and the above specific functions. Despite, this lack of knowledge, coelomocytes are known as the most actively involved elements during the repair phase of asteroid arm regeneration.

CF Franco et al Proteomics (2011) 11: 1359-1364; CF Franco et al Proteomics (2011) 11: 3587-92; CF Franco et al Electrophoresis (2012), 33, 3764–3778; C Franco et al J Proteomics (2014), 99: 1-25; C Franco et al Proteomics (2013), 13, 686-709 (Review article)

**OBJECTIVES**

Coelomocyte populations will be first characterized by flow cytometry and microscopy. These results will be used to define sorting strategies by flow cytometry, in order to perform their proteome characterization. These tools will be used to follow coelomocytes circulating dynamics during regeneration. Additionally, identified specific biomarkers for each population during this task will allow to generate probes for flow cytometry and *in situ* microscopy localization of tissue localized coelomocytes.

1- Identification and functional characterization of coelomocyte populations of starfish M. glacilais.
2- Definition of specific molecular biomarkers for each coelomocyte population
3- Identify and characterize coelomic cell sub-populations involved in regeneration conditions.
4- Sort cell sub-populations present in non- and regenerating conditions.

Depict proteome dynamics of coelomocyte populations

**PROJECT DESCRIPTION**

The process of sea-stars handling, including the induction of regeneration and tissue collection, will be made at the Aquário Vasco da Gama (Dafundo, Oeiras). Protocols are being developped for flow cytometry characterization of coelomocytes populations at Instituto Gulbenkian de Ciências (Oeiras).

Task 1: Characterization of cell sub-populations by flow-cytometry and microscopy will be performed on the following parameters: cell cycle analysis, cell proliferation, cell viability, apoptosis assays and phagocytic activity.
Task 2: Cell populations individually sorted by flow-cytometry will be used for proteome characterization by LC-MSMS, aiming to extend the functional knowledge on these cells and their potential involvement in regeneration.
Task 3: Sorted coelomocyte population protein extracts for each regeneration time-point will be obtained, quantified and digested with trypsin for differential LC-MS/MS analysis. Differentially expressed proteins between different groups will be quantified and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics.
Task 4:Protein identification and quantification data treatment: Profile data from the MS scans are transformed to *m/z* peak lists. Protein identification will be performed using the MS/MS spectra and submitting to a search engine using a general database. For quantification, all reimported peptides of an identified protein are included, and the total cumulative abundance is calculated by summing the abundances of all peptides allocated to the respective protein.
Task 5: Pathway analysis using the proteins identified with expression differences between each pair of sample groups
Task 6: Writing of Master Thesis