Dissertation Project – 2nd Cycle

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TITLE: Advanced cell-based biosensors for detection of label-free viral pathogens

BACKGROUND

Infectious pathogens pose serious health problems. Indeed, infectious diseases kill more people worldwide than any other single cause. Immunization is among the most effective intervention in modern medicine, reducing global mortality rates caused by infectious diseases by approximately 3 million people per year. Despite this progress vaccines have not yet realize their full potential since (1) they are not often available in developing countries and (2) effective vaccines have not yet been developed for several human diseases. A second arm of defense is the use of antiviral drugs. These are not only essential to fight diseases for which no vaccines are available, as they are more efficient in stopping an infection that has already started.

However, development of effective prophylactic or therapeutic agents against viral pathogens faces a major difficulty: accurate and reliable detection and quantification of infectious virus. Current methods fail to provide it, meeting at least one of the following pitfalls: extremely time-consuming, lack high-throughput potential, based on indirect measurements, or require the use of reporter genes (label-containing virus). These are the limitations this project will overcome by developing novel mammalian cell-based biosensors for the detection and quantification of label-free human viruses.

OBJECTIVES

The aim of the work herein proposed is to develop and apply mammalian cell-based biosensors for detection and quantification of human viral pathogens. Briefly, mammalian cell lines will be modified by means of molecular biology tools and cell engineering in order to express these sensors. However, they will only be activated upon virus infection - triggerable system - originating a signal easily detected (like fluorescence emission).

In the end, these cell-based virus biosensors will establish a new platform for diagnostic and clinical applications and basic virology research, enabling the development of novel virus therapeutics.

PROJECT DESCRIPTION

Work Plan:

Task 1 – Learning animal cell culture techniques: working in laminar flow, propagation of cells, determining cell concentration, long-term storage of cells. Reading work-related bibliography.

Task 2 – Learning molecular biology methodologies: construction and cloning of plasmids to express the sensors required for the work. Transfection of mammalian cells, cell engineering and analysis of cells by flow cytometry and fluorescence microscopy.

Task 3 – Learning the techniques required for recombinant virus manipulation: culture of producer cells and production of recombinant adenovirus and lentivirus in tissue flask, quantification of infectious and total particles. Characterization of cell growth and viral production. Methods: flow cytometry, RT-qPCR, Nanosight, plaque assay, TCID50 and Western-Blot.

Task 4 – Characterization and comparison of different cell-based sensors. Analysis of expression of the sensor (e.g. PCR, Western-Blot), background (noise) and signal generated after viral infection. Further optimization of the sensor cells lines according with the results. Final characterization – optimization of infection conditions, sensor sensitivity and detection kinetics – and validation of the sensor.

Thesis – Master thesis writing.

Recommended references:

Rodrigues AF, Soares HR, Guerreiro MR, Alves PM, Coroadinha AS (2015) Viral vaccines and their manufacturing cell substrates: New trends and designs in modern vaccinology. Biotechnol J.10(9):1329-44. http://dx.doi.org/10.1002/biot.201400387

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										