STUDY OF CENTRAL CARBON METABOLISM OF ENDOTHELIAL CELLS IN NORMAL AND PATHOLOGICAL CONDITIONS

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Introduction

Endothelial dysfunction is a primary factor in the onset and progression of atherosclerosis and other vascular related diseases as well as in the thrombus formation and stabilization. Not just the size but rather the stability of atherosclerotic plaques is a determinant for acute clinical implications.

Emerging evidences indicate that pathological blood vessel responses and dysfunctionality of Endothelial Cells (ECs) are associated with metabolic alterations in ECs. Preliminary data from our group have suggested that ECs derived from patients show an altered hyperproliferative phenotype and a resistance to apoptosis when compared to controls.

In this study we aim to establish an *in vitro* model of endothelial pathology, using patient-derived endothelial cell lines which are subjected to a systematic evaluation against control cells in order to determine the metabolic profile and to understand if endothelial dysfunctionality is a consequence of an abnormal endothelial cell metabolism.

The endothelial cell lines are studied in terms of morphology, proliferation and metabolic profiling.

Overview

Acute myocardial infarction

Acute myocardial infarction is a leading cause of morbidity and mortality worldwide.

It occurs when myocardial ischemia, a diminished blood supply to the heart, exceeds a critical threshold and overcomes myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis.

An interruption in the supply of myocardial oxygen and nutrients occurs when a thrombus is superimposed on an ulcerated or unstable atherosclerotic plaque and results in coronary occlusion.

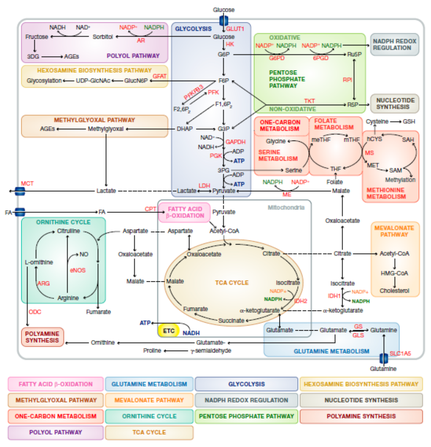
An *in vitro* model of endothelial pathology from patients undergoing treatment for acute myocardial infarction has been developed and studied.

Endothelial Cells metabolism

Endothelial cells (ECs) are key players in health, as well as in life-threatening vascular diseases.

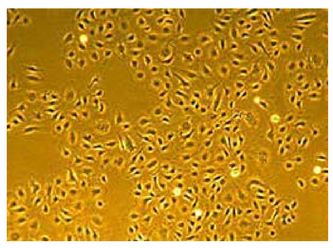
Endothelial dysfunction is a primary factor in the onset and progression of arteriosclerotic vascular disease (ASVD) unbalancing vascular homeostasis and also predisposing thrombus formation.

ECs have high glycolytic activity and the glycolytic flux higher than glucose, fatty acid and glutamine oxidation, resulting in the generation of >85% of the total cellular ATP content.

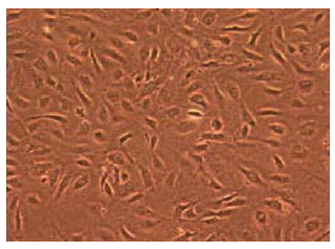


Results

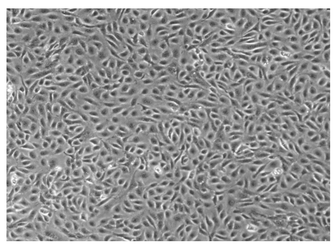
Cells phenotype



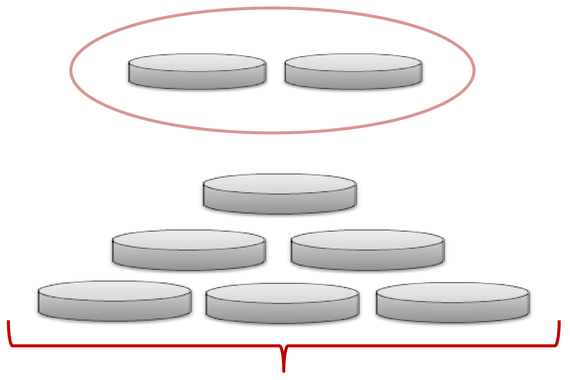
HUVEC cells



HCAEC cells



Patient cells



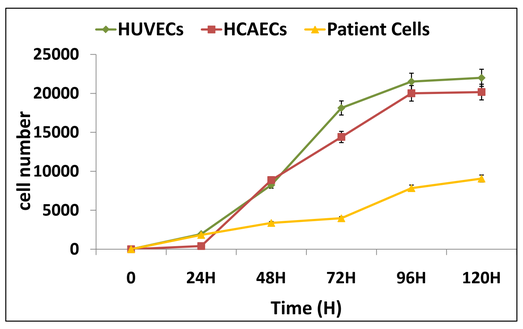
Control Cell lines:

- **Human Coronary and Artery Endothelial Cells** (HCAEC)
- **Human Umbilical Vein Endothelial Cells** (HUVEC)

Patient Cell lines:

- Endothelial Cell lines from thrombus extracted from patients undergoing treatment for Acute Myocardial Infarction

Cells proliferation



The profile of the growth curve shows that both control cells, HCAECs and HUVECs, have a higher proliferation rate than Patient Cells.

Doubling Time:

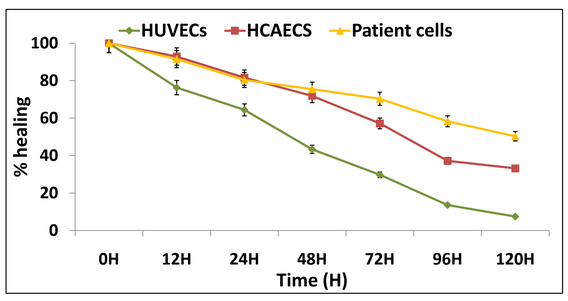
HUVECs	HCAECs	Patient Cells
t_d=25H	t_d=36H	t_d=84H

Cell migration ability

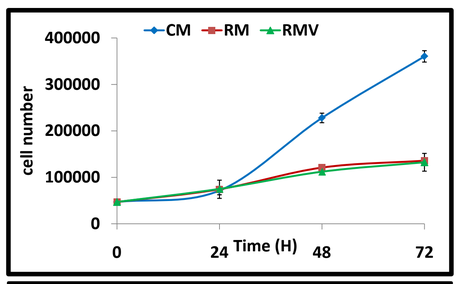
Wound Healing assay

Patient cells migration ability is slower than the commercially controls, in fact at 120H the wound is almost 60% opened.

HUVEC cells migrate quickly than the other cellular models studied.



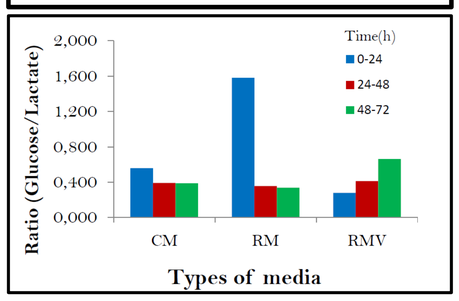
Biochemical analysis in HUVEC cells

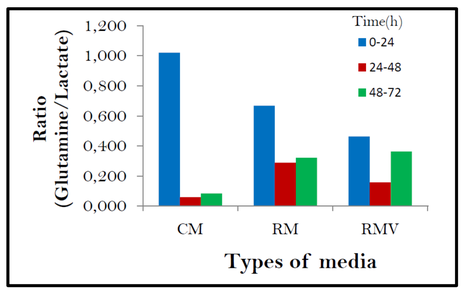


In order to understand and investigate the difference in proliferation among the distinct models of ECs, we performed metabolic measurements in different media conditions.

First experiments have been performed in HUVEC cells model.

CM – Complete medium: EGM Bulletkit, Lonza;
RM – Restricted medium, 2% Serum;
RMV – RM with VEGF





The concentrations of glucose and lactate were measured spectrophotometrically from the cell media using the chemical analyzer Cobas Mira (Roche Applied Science).

Their consumption and production rates, **Kpc**, were determined using:

$$Kpc = \frac{\Delta M}{N_f - N_o} \times \mu \qquad \mu = \frac{\ln\left(\frac{N_f}{N_o}\right)}{t_f}$$

$\Delta M = M_f - M_o$: moles of metabolite produced or consumed

Final remarks

The research aims at performing a complete characterization of the metabolic profiles of endothelial cells (ECs) in normal and pathological conditions, and will include an integrated systems biology approach and proteomic analysis.

The recent work showed here is a part of this attempt, and includes a preliminary study of cells growth and of its ability in migration. This study will allow the identification of the insights and causes that result to dysfunctional ECs and the metabolic alteration related to EC dysfunctionality.

The achievement of a novel and improved therapeutic strategy against the EC dysfunctionality associated to acute myocardial infarction, is one of the principal aim of this project.

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