

## PROJECT PROPOSAL

### 1. Name, telephone number, email, address and professional status of supervisor and co-supervisor

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### 2. Project title

*Development of vessels-on-a-chip for the study of thrombosis*

### 3. Short background

It is now every time more common to use the so called “organ-on-a-chip” structures to study in vitro the function and behaviour of different diseases and cell-mechanisms in a set-up that is more close to the physiological conditions than the more traditional in vitro cell work. The organ-on-a-chip are microchips that recapitulate the microarchitecture and functions of living organs, such as the lung, heart, and intestine.

In this project we want to create a chip focusing our research on the effects of silencing von Willebrand Factor, which is a well-known determinant protein that plays an essential role in hemostasis by controlling the adhesion and aggregation of platelets at sites of vascular injury.

### 4. Aims

The aim of the project is to establish a microchannel system that can mimic a vessel, and that can be used to assess the effect of particular candidate genes on the thrombus formation.

### 5. Methods

To achieve our aims, we have created some microvessel chip models in collaboration with University of Uppsala. We will coat these microvessels with endothelial cells and we will use gene-silencing to down-regulate key genes that modulate risk of thrombosis. We will first silence vWF in endothelial cells in our “vessel-on-a-chip” model as a positive control, and check platelet attachment under flow conditions as proof of principle that will be essential to test future gene-silencing of candidate genes with unknown function.

With this project we expect to be able to answer the following questions:

- Is it possible to develop a technique to silence cells in the chip?
- Are these results obtained from the chip comparable to those on cultured cells in 6-well plates?
- Can we establish a reliable system to test the effect of unknown genes in the coagulation process?

### 6. Working plan, time plan, dates to start and end the project

We are flexible but would prefer to start the project as soon as possible.

## **7. Short method description**

-cell culture: we will use HUVEC cells and EA.hy.926 endothelial cells and we will culture them in parallel in traditional 6-well plates and into microvessels. For that, cells will be grown in the appropriate medium and supplemented with 10% Fetal Bovine Serum (FBS) and will be seeded into the microchips at a of 1.5 – 1.6 million cells per ml for chip experiments. Prior to cell seeding, chips will be coated with gelatin to allow better attachment of the cells.

-gene silencing and development of a novel technique to silence and culture endothelial cells in micro vessels: gene silencing will be done by siRNA transfection using Lipofectamine. Silencing will be performed in 48-well plates according to recommendations of the provider. After 24 hours, cells will be trypsinised and seeded into the microchip.

-Citrated whole blood or platelet rich plasma containing labeled platelets will be perfused through the microchannel and the attachment of platelets in the endothelium will be measured for both control and silenced coated microchannels.

-RNA extraction and quantification by real-time PCR both in the chip and in cultured cells