DEVELOPMENT OF A MICROFLUIDIC BIOMIMETIC DEVICE FOR TRIPLE NEGATIVE BREAST CANCER STEM CELLS EXTRAVASATION STUDIES

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1. Introduction

Since several years, the discovery of a population of CSCs within tumors has altered our conception of cancer organization and treatment responses [1]. CSCs are suspected of being involved in the metastatic cascade that results in the emergence of distant secondary tumors. Interestingly, the most aggressive of cancers, the triple negative breast cancers (TNBC) is the subtype containing the highest amount of CSC [2]. We want to assess if metastases arise from circulating CSCs able to extravasate and resume growth and/or if metastases arise from 'non-CSC' Circulating Tumor Cells (CTC) that will reprogram as CSCs once extravasated in appropriate environment [3].

2. Microfluidic device

This device, fabricated in silicone elastomer (PDMS), is composed of two chambers. The first is dedicated to reproduce a microvasculature network mimicking the configuration of natural blood vessels [4] and the second to generate a specific modifiable metastatic niche or to apply the chemo-attraction stroma (Fig.1). In the top layer, the entrance channel is 800 µm wide. Each channel, of 50 µm of deep, are divided into two half-width channels, up to 8 channels of 100 µm in the center of the device. The bottom layer is composed of a channel, 1mm wide and 150 µm thick placed under each top channel. These chamber layers are separated by SU8 porous membrane with pores sizes of 10 µm (Fig.2).

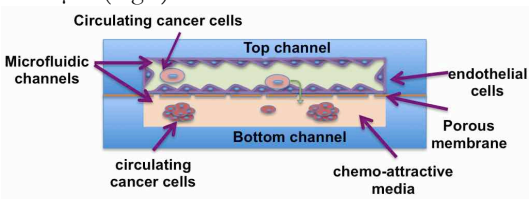

Figure 1: Cross section of the microfluidic device

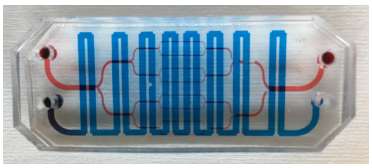

Figure 2. Photo of the microfluidic PDMS device filled with red ink (top channel) and blue ink (bottom channels).

3. Results

We have successfully generated 3D biomimetic blood vessels in a reproducible manner. Briefly, channels inner surface are coated with fibronectin (50 µg/ml) and collagen (100 µg/ml). Then, Human Umbilical Vein Endothelial Cells (HUVECs) are injected at high density (8.105 cells in 15 µl) in the top chamber and cell culture medium (EGM-2) is perfused during 3 days to allow for cells adhesion and proliferation (Fig.3). We have also evaluated cancer cell attachment on HUVECs monolayer. For this, HUVECs monolayer was first activated with 10 ng/ml TNF-Alpha for 24 h. Then, human breast adenocarcinoma cells (MDA-MB-231) were injected inside the top channel under flow (2 to 10 µl/min). Our preliminary results showed that a sub-population of cells remained attached to the activated endothelial monolayer (Fig.4).

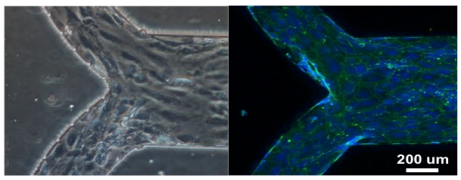

Figure 3. Image of transmission microscopy in phase contrast (left). Fluorescence microscopy image (right): Hoechst staining (blue) and anti-CD31 staining (green)

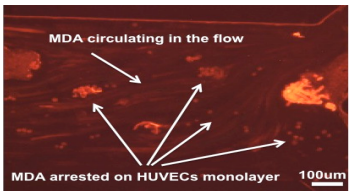

Figure 4. MDA cells perfused activated HUVECs monolayer

Our next step is to keep media flowing to long-term study attached cells extravasating and collect extravasated cells through the bottom channels and non-extravasated cancer cells through the upper channels in order to study their phenotype (CSC). Our ultimate goal is to modify bottom chamber environment and identify its impacts on extravasation, CSC phenotype and ability to develop secondary tumors.

References

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