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Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2024023

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Experiment title	Placenta-On-Chip: modeling the trophoblast-stroma crosstalk using time-lapse microscopy brightfield imaging and machine learning analysis
MRF Instrument	**TLM platform**
Access Route	Direct Access
Science Areas	Biology and Bio-materials, Engineering
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 5
Previous GP Number: -
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

Understanding the physiological processes at the early stage of pregnancy is a challenge in reproduction research. A successful pregnancy is related to the establishment of implantation of the embryo to the maternal endometrium. In this phase, the fetal extravillous trophoblast (EVTs) migration and invasion into the maternal uterus is a crucial event and is regulated by diverse cells of the maternal environment. Since trophoblast cells give rise to the placenta, alteration in their invasion through the endometrial tissue leads to dysfunction in placentation leading to pathological pregnancy. In this activity, we propose a model based on organ-on-chip (OOC) technology, namely placenta-on-chip to recreate the early placentation process by modeling the fetal-maternal interface with the trophoblast migration (2D) and invasion toward the stromal cells (3D). The extremely delicate biological scenario requires a minimally invasive methodology to investigate such phenomena over time.

Publications -

Sample record sheet

Principal contact Professor Eugenio Martinelli, University of Rome Tor Vergata, ITALY
MRF Instrument **TLM platform** **Days Requested:** 5
Special requirements:

SAMPLE

Material	cells in the culture media	-	-
Formula	-	-	-
Forms	Liquid		
Volume	10 ml		
Weight	mg		
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	- K	-	-
Pressure Range	- mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	-	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	-	-

1. Background and Context

Understanding the physiological processes at the early stage of pregnancy is a challenge in reproduction research. A successful pregnancy is related to the establishment of implantation of the embryo to the maternal endometrium. In this phase, the fetal extravillous trophoblast (EVTs) migration and invasion into the maternal uterus is a crucial event and is regulated by diverse cells of the maternal environment such as endothelial cells, immune cells, and stromal cells[1]. Since trophoblast cells give rise to the placenta, alteration in their invasion through the endometrial tissue leads to dysfunction in placentation leading to pathological pregnancy. In this activity, we propose a model based on organ-on-chip (OOC) technology, namely Placenta on Chip (POC) to recreate the early placentation process by modeling the fetal-maternal interface with the trophoblast migration (2D) and invasion toward the stromal cells (3D). The extremely delicate biological scenario requires a minimally invasive methodology to investigate such phenomena over time.

The group of prof. Campagnolo has a wider experience in the proposed field publishing several studies about the physio-pathological processes involved in the early stage of pregnancy. Among them, they have focused attention on specific molecular mechanisms regulating the interaction between the maternal-fetal interface[2–4], revealing how alteration of specific gene expression, and related signal transduction pathways, can lead to placentopathies. Moreover, they also investigated the effect of different engineered nanoparticles on placental and fetal development showing embryotoxicity in “in vivo” experiments.

Students (master's thesis and PhD students) and a postdoctoral researcher are working on different fields related to this study.

2. Proposed experiment

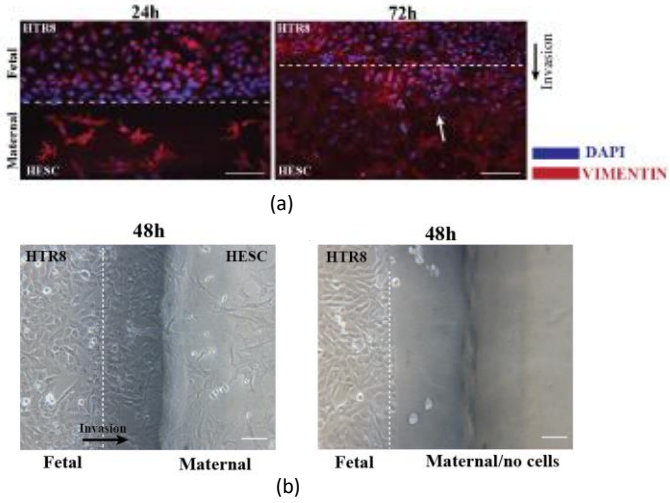
In light of this, the facility offered by Prof. Martinelli includes LOC fabrication on demand, Time-Lapse-Microscopy for brightfield video acquisition, incubator, and lastly, not less important, video analysis through machine learning algorithms for the quantitative evaluation of the outcomes such as migration ability. We will culture that trophoblast cells (HTR8) in close proximity to the extracellular matrix (ECM) with and without stromal cells (HESC), to evaluate the promotion of the attraction effect of HESC cells to HTR8 in healthy conditions. We’ll plan to compare the effect on cell migration and invasion with the case of specific gene silencing. By getting access to the facility of the time-lapse microscopy, we would have the possibility to perform label-free time-lapse microscopy acquisition for 24hr and 48hr to evaluate the dynamics of the migration in the two opposite conditions. Thanks to the facility, we will achieve LoC devices on demands, specifically tailored on the experimental conditions, and the video analysis through dedicated machine learning and image analysis algorithms. Previous expertise of the facility LAB group Prof. Martinelli, assure the reliability of the analysis and of the achievable outcomes in terms of migration capability morphodynamics of moving cells, etc.

Why ISIS@MACH ITALIA instruments are needed

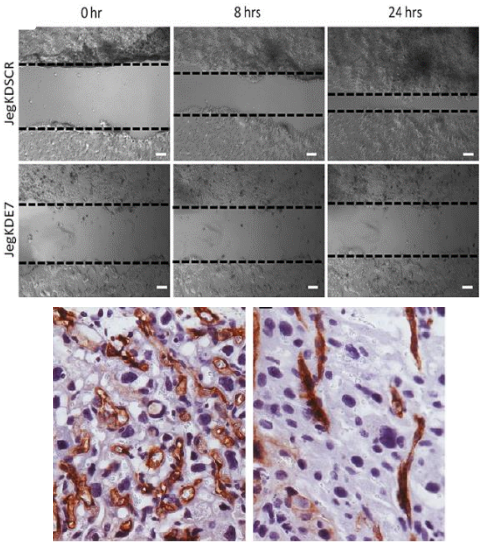
- POC device -> will allow a dedicated device for the specific experiment to assure cell viability for the time of acquisition (60hr)
- TLM facility -> will allow us to observe the dynamics of the migration phenomenon in label-free modality, therefore minimally invading the delicate biological environment through the use of cellular staining (Fig.1a). This will add incredible value with respect to the endpoint analysis.
- ML and Video Analysis -> will allow to quantitatively evaluate the dynamics of migration either in terms of cell shape and cell kinematics characteristics, with the additional opportunity to investigate transient phenomena not visible and long-term checkpoint.
- Analysis will be performed through the use of image segmentation algorithms, particle velocimetry algorithms, and eventually deep learning architectures. The explainability of the architectures will be always provided.

3. Summary of previous experimental proposals or characterisation

Among their studies, the group of xxx has investigated the cross-talk between the maternal-fetal interface by studying the correlation between the anomalous expression of the epidermal growth factor-like domain 7 (EGFL7) and the poor reproductive outcome[2,4]. As an example, in Figure 1a the wound healing assay shows the reduction of migration of trophoblast Jeg3 cells (fetal side) after the downregulation of EGFL7 gene expression[5]. Moreover, different studies “in vivo” and “in vitro” have been focused on the development of embryotoxicity due to the exposure to different compounds, such as the oxidized single-wall carbon nanotubes (SWCNTs)[6], and engineered nanoparticles (NP) like Ag, ZnO, TiO_2, and SiO_2[7–9]. For example, the immuno-histochemical analysis showed decreased vascularization in malformed mice’s placentas due to the presence of reactive oxygen species (ROS) (Figure 1b).



a)



b)

4. Justification of experimental time requested

We’ll plan to run two sessions of experiments, for a total of 5 days (2.5 days a session, 60hrs). Each session will be devoted to a distinct condition (control vs gene silencing) and will be divided into parallel experiments run on distinct compartments of the POC. Replicas as well different environment set-up (HESC or not) will be performed in each compartment in order to enlarge the reliability and the reproducibility of the outcomes.

References

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