CMI-NEN Booster Project

Project Title

Heart-on-chip to measure cardiac P-glycoprotein function using [^{18}F]MC225 and PET imaging

Team Members & Biography

Drs. P. Mossel, MD, Phd-Student, Department of Nuclear Medicine, University Medical Center Groningen. Pascalle Mossel is a medical doctor and Phd student under supervision of **dr. G. Luurtsema** at the department of Nuclear Medicine and Molecular Imaging at the University Medical Center in Groningen. Her research focus is on the evaluation of the P-glycoprotein function and blood-brain barrier integrity using a novel PET tracer, [^{18}F]MC225.
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Dr. G. Luurtsema, Associate Professor in Clinical Radiochemistry, Department of Nuclear Medicine, University Medical Center Groningen. Gert is focused on a broad range of topics, ranging from tracer development to validation of clinical imaging applications. With support of NWO-STW funding, he has accomplished a research line on imaging of the blood-brain barrier (BBB) integrity in relation to brain disorders and drug delivery. Besides this, he is supervising > 7 PhD students, 5 master students and 3 postdocs. Currently, he is (co-)author of more than 100 peer-reviewed scientific articles in high ranking journals and scientific textbooks. He was guest editor of the journals Current Pharmaceutical Design and Contrast Media & Molecular Imaging.

Dr. A.D. van der Meer, Associate Professor in the department of Applied Stem Cell Technologies at the Faculty of Science and Technology, TechMed Centre, University of Twente. Andries heads an independent research team within the Applied Stem Cell Technologies department of the Bioengineering Technologies cluster, supervising five Ph.D. candidates and coordinating multiple national and European research projects. The research of his team is focused on the development of organs-on-chips, which are stem cell-derived, microengineered models of the human body that are used to model diseases and discover new treatments.

Dr. V. Schwach, Post-doc in the department of Applied Stem Cell Technologies at the Faculty of Science and Technology, TechMed Centre, University of Twente. Verena is working on the differentiation of human pluripotent stem cells towards cardiac cells, including cardiomyocytes and endothelial cells for disease modeling and cardiotoxicity screening.

Prof. dr. P.C.J.J. Passier, Head of the department of Applied Stem Cell Technologies at the Faculty of Science and Technology, TechMed Centre, University of Twente Professor Anatomy and Embryology, stem cell biology, in particular applied stem cell technology

Key words

- PET Imaging
- Heart-on-chip
- P-glycoprotein
- Pharmacokinetics
- Cardio-vascular agents

Clinical Relevance

The pharmacological effect of a drug depends on the availability at the site of action and is affected by processes of absorption, distribution, metabolism, and elimination. Transporters at the target site, responsible for the influx and efflux of drugs in and out of cells influence the intracellular drug concentrations. P-glycoprotein (P-gp) is a plasma membrane protein and efflux transporter that is involved in the transport of a wide variety of structurally unrelated compounds (P-gp substrates) out of the cell. P-gp is located in several organs, including the heart. It plays an important role in the protection of these organs against potentially toxic compounds, including pharmaceuticals, by regulation of drug absorption and excretion. The majority of prescribed cardiovascular agents (e.g., antiarrhythmic agents, anticoagulant agents) are P-gp substrates and therefore it is evident that P-gp plays an important role in the drug availability at the target site.[1] Variations in genotype and physiological changes (including ageing) can lead to variable expression of P-gp in the human heart. Also, drugs can have an inhibitory or inducing effect at P-gp, potentially leading to drug-drug interactions, overdosing and potential side effects. An increase in P-gp function can lead to lower efficacy in cardiovascular treatment, when the administered drug is a P-gp substrate. On the other hand, decreases in P-gp function can lead to unwanted side effects, relative overdosing, and cardiotoxicity, as uptake of pharmaceuticals in the myocard is higher. Adequate measurements of the P-gp function *in vivo* would allow for an increased understanding in the pathophysiological mechanisms underlying these changes in bio-availability. [^{18}F]MC225 has proven to be a suitable tracer to measure changes in P-gp function *in vivo*, at least for cerebral P-gp function with PET (Figure 1).[2,3] The aim of this study is to validate the use of an organ-on-chip model for measuring cardiac P-gp function. To this aim we study and compare the expression and function of P-gp both in an heart-on-chip model and in a preclinical animal model using [^{18}F]MC225.

Challenge

Nowadays preclinical animal studies are necessary to study the specificity and sensitivity of new PET tracers for a certain target *in vivo.* However, animal models may not accurate represent the physiology of the human heart, since the expression of P-gp transporters might be different in between human and animal models. By developing a 3D organ-on-chip model of the heart, so-called engineered heart tissues (EHT) containing both human pluripotent stem cell (hPSC)-derived vascular endothelial cells and hPSC-derived cardiac myocytes, the physiological conditions in the human heart are simulated (Figure 2), allowing for realistic measurements of cardiac P-gp function without the use of animal models.[4] This will be the first time an organ-on-chip will have a role in the evaluation of a novel PET tracer. This research project paves the way to future projects in which the effect of P-gp function at the efficacy and safety of cardiovascular agents can be studied in organ-on-chip models instead of laboratory animals.

Strategy and time-line

Project	**Time schedule**
Heart-on-chip with engineered heart tissues (EHTs)	Analysis immunofluorescence staining – 6 months Analysis using [^{18}F]MC225 – 6 months
Preclinical animal study: measuring cardiac P-gp function *in vivo*	Writing and approval IVD - 3 months Micro-PET scans – 6 months Data analysis – 3 months

Synergy

For answering the research question a collaboration was set up between the University Medical Center Groningen and the University of Twente. The aim of this study is to evaluate the value of an organ-on-chip model in preclinical PET studies by comparing [^{18}F[MC225 tracer uptake in an animal model with outcomes of heart-on-chip imaging. Positive results of these organ-on-chip studies will set the stage for implementation of a combination of organ-on-chip and PET imaging in drug development, as it would be creating the possibility to study pharmacokinetics of new pharmaceuticals without the use of laboratory animals. Cardiotoxicity and bio-availability screening *in vitro* at an organ-on-chip model may be an important feature in the evaluation of novel cardiovascular agents.

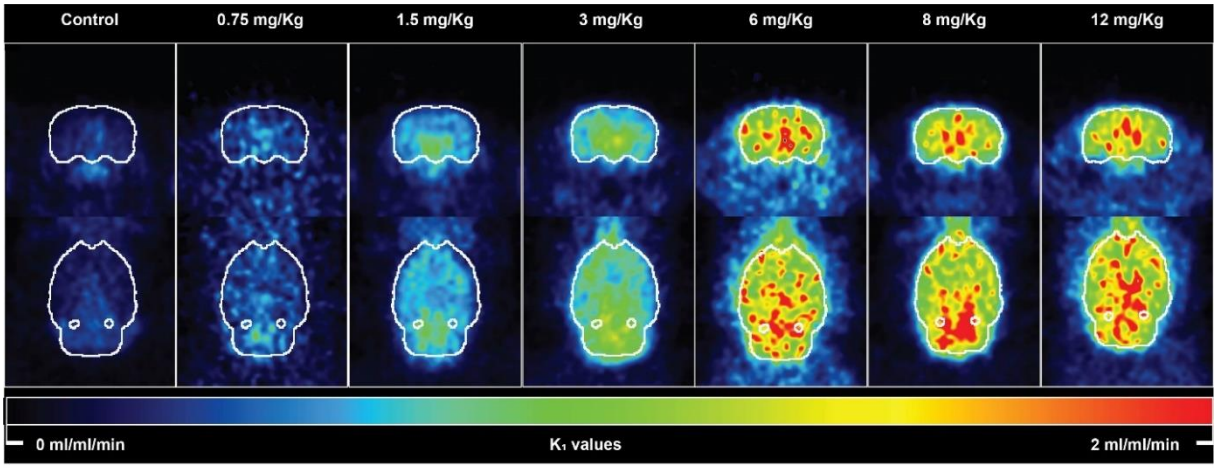

Figure 1 – Preclinical dose response study to explore the sensitivity of [^{18}f]MC225 for small changes in cerebral P-glycoprotein function.

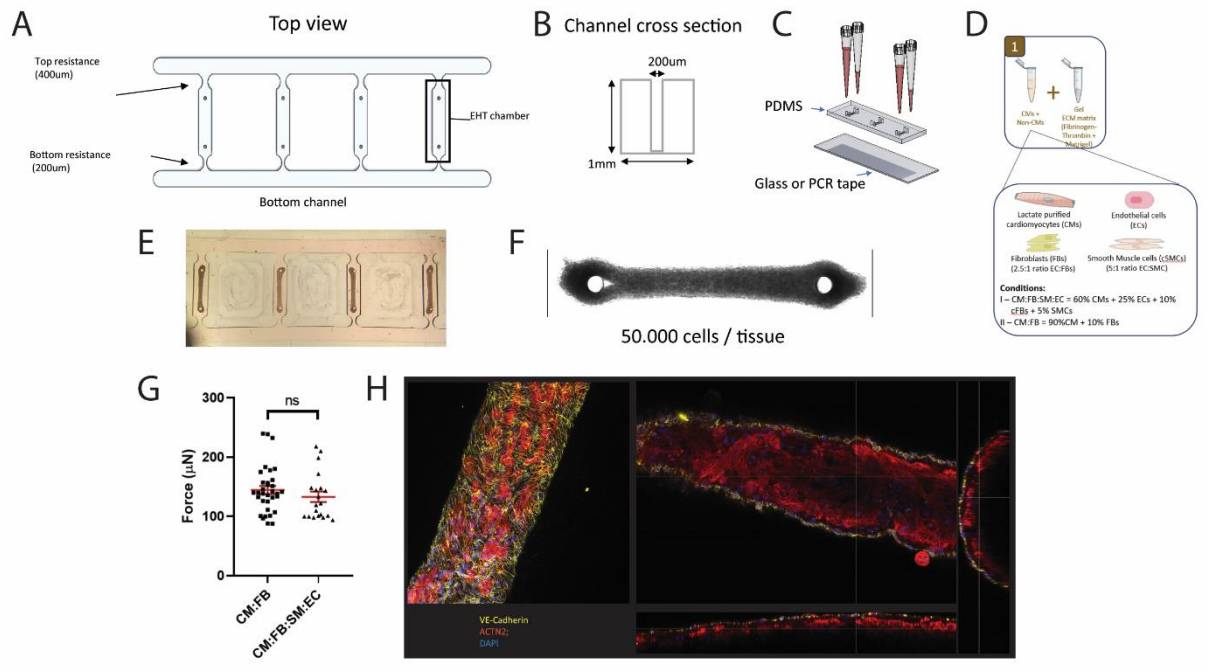

Figure 2: µ-Engineered Heart Tissue (EHT) platform. A) Top view of heart-on-chip model with EHT chamber. **B)** Cross section of EHT chamber. **C)** Overview of the chip assembly. **D)** Cell types included in the EHT. **E)** Overview picture of the full chip with four EHTs. **F)** Single EHT tissue with 50,000 cardiomyocytes. **G)** Absolute force of contraction quantified in EHTs with 1) CM:FB only cardiomyocytes (CMs) and cardiac fibroblasts (FBs) and 2) CM:FB:SM:EC cardiomyocytes (CMs), cardiac fibroblasts, cardiac smooth muscle cells (SM) and endothelial cells (EC). **H)** Immunostaining of EHT with VE-Caderin for ECs, alpha- actinin (ACTN2) for cardiomyocytes and DAPI for the cell nuclei.

<u>Envisaged outcome</u>

If indeed, cardiac P-gp function can be measured using an heart-on-chip model, in future research the effects of alterations in P-gp function on the efficacy of the cardiovascular agents can be studied in order to establish an accurate prescription dose.

<u>Impact on healthcare</u>

A better understanding of the differences and consequent challenges of treating and diagnosing cardiovascular disease is essential in improving public health. Positive outcomes of this pilot study will open the gate to larger funding options for drug development, with a special focus on the implementation of organ-on-chip models to study bioavailability and toxicity of novel pharmaceuticals. Further funding options include PUSH funding of Siemens Healthineers or national funding of the Hartstichting.

<u>Requirements and budget plan</u>

Project	**Facilities/Materials**	**Costs**
Heart-on-a-chip implementation	Cell culture medium, growth factors, maturation factors etc.	8000,-
Cardiac P-glycoprotein function, Preclinical study: Scan costs Micro-PET	10 scans Micro-PET	10x600=6000,-
Travel costs		1000,-
Total		**15000,-**

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