DELIVERY SYSTEMS: FROM MICROFLUIDICS TO NANOTECHNOLOGIES

Room:	**Aalmarktzaal**
Time:	**Monday 11:00 to 12:30**
Chair:	**Amalia Dolga**
Organizing FIGON-partner(s):	**NVF, NVT, ZonMW**

Invited lectures:

11:00 – 11:30 **3D Vessels-on-chip to model vascular disease and beyond**
Dr. Valeria V Orlova, Leiden University Medical Center (LUMC)

11:30 – 11:50 **Pharmacomicrobiomics: a novel route towards personalized medicine**
Dr. Jingyuan Fu, University Medical Center Groningen

Selected abstracts:

11:50 – 12:10 **Dissecting how cells internalize and process nano-sized drug carriers for nanomedicine application**
Prof. Anna Salvati, University of Groningen

12:10 – 12:20 **Anionic lipid nanoparticles preferentially targeting mRNA to hepatic RES *in vivo***
Gabriela Arias-Alpizar, Leiden University

12:20 – 12:30 **A lipid nanoparticle RNA vaccine platform for induction of antigen-specific tolerance**
T. Fariaby, Leiden University

Indicated speaker time includes 3-5 minutes for discussion

NVF Dutch Pharmacological Society NVT TOXICOLOGIE ZonMw

3D Vessels-on-chip to model vascular disease and beyond

Valeria V Orlova, Leiden University Medical Center (LUMC), Leiden, The Netherlands

Small vessel diseases are the leading cause of disability and death worldwide. The major challenge is that they are multisystem disorders affecting different organs, such as the brain, heart and kidney. They have been difficult to model *in vitro* because high-quality vascular cells are difficult to derive from patients and the local organ microenvironment which is difficult to mimic often contributes to the disease. For this reason, human induced pluripotent stem cells (hiPSCs) have become attractive sources of patient- and organ-specific cells. We use hiPSCs to re-create blood vessels on microfluidic chips that recapitulate micro- and macrovascular networks and the local microenvironment. We developed efficient protocols to differentiate hiPSCs towards ECs, pericytes/vSMCs, and inflammatory cells (monocytes and pro- and anti-inflammatory macrophages). We have demonstrated that both micro- (10-50 μm) and macro-scale (250-300 μm) perfusable 3D vessels composed of hiPSC-derived endothelial cells, pericytes/vSMCs, and other non-vascular components, such as hiPSC- derived astrocytes, can be generated inside the microfluidic devices. Recently we also developed a microphysiological system that behaves as a human "mini-heart" using cardiomyocytes, endothelial cells, and cardiac fibroblasts all derived from hiPSCs. These mini-hearts can be produced just 5000 cells and without specialized equipment. They thus represent a low-cost, low tech platform for cardiac drug discovery and disease modeling. Using isogenic patient hiPSC lines and 3D vessels-on-chip, we recapitulated the phenotype of a genetic vascular disease called hereditary hemorrhagic telangiectasia (HHT). This patient-based hiPSC model serves as proof of principle that vascular diseases can be modeled using patient-specific hiPSCs in 3D microfluidic chips and used to identify new target cells and possible pathways for therapy.

Pharmacomicrobiomics: a novel route towards personalized medicine

Jingyuan Fu, PhD, University Medical Center Groningen (E-mail: j.fu@umcg.nl)

Inter-individual heterogeneity in drug response is a serious problem that affects the patient's wellbeing and poses enormous clinical and financial burdens on a societal level. Pharmacogenomics has been at the forefront of research into the impact of individual genetic background on drug response variability or drug toxicity, and recently the gut microbiome, which has also been called the second genome, has been recognized as an important player in this respect. Moreover, the microbiome is a very attractive target for improving drug efficacy and safety due to the opportunities to manipulate its composition. Pharmacomicrobiomics is an emerging field that investigates the interplay of microbiome variation and drugs response and disposition (absorption, distribution, metabolism and excretion). To identify microbial factors involved in drug metabolism and obtain mechanistic understanding of host-microbe interaction in drug metabolism, we combine the large-scale human cohort study and pharmacokinetics analysis on the innovative, personalized organ-on-a-chip (including both intestine- and liver-on-a-chip). Our study will lay the foundation for major advances in personalized medicine.

Dissecting how cells internalize and process nano-sized drug carriers for nanomedicine applications

A. Salvati[1]
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Introduction

Nano-sized materials are used in nanomedicine to deliver drugs more efficiently to their site of action. In order to improve their efficacy and fully exploit nanomedicine potential, a better understanding of how cells internalize and process nano-sized materials is required.

Objectives

Within this context, our research is focused on characterizing the molecular details of the early interactions and recognition of nano-sized materials at the cell membrane, and the subsequent mechanisms of uptake and intracellular trafficking.

Methods

To this aim, we combine classic transport studies with inhibitors[1] and RNA interference to genetic screening and proteomic-based methods. Additional efforts are focused on developing *in vitro* endothelial cell barriers more closely resembling the barriers nanomedicines encounter *in vivo*.[2] Uptake and distribution of nanoparticles have also been studied in tissue slices from the major organs in which nanoparticles distribute, such as the liver, lungs and kidneys, as an advanced *ex vivo* 3D model. Intracellular trafficking kinetics are determined via fluorescence imaging in live cells and a new method we developed based on organelle flow cytometry.[3,4]

Results and Conclusions

Our results show that the same cells process nanoparticles in different ways when they are developed into an endothelial cell barrier rather than at different degrees of cell density, as commonly applied for *in vitro* studies.[2] Furthermore we show that nanoparticles are internalized *ex vivo* in liver tissue slices, and within the tissue slices, higher uptake is observed in the Kupffer cells, as it is observed also *in vivo*.[5] Additionally, we found that the corona molecules adsorbing on the nanoparticle surface once applied in serum can interact with specific cell receptors and in this way they also affect the mechanism cells use for their internalization.[6] Based on these findings, we used liposomes of different composition to form different coronas and identify corona proteins associated with higher or lower uptake by cells.[7] Thus, nanocarrier design can be tuned in order to modulate the corona forming in serum and in this way affect cell receptor interactions and nanocarrier uptake efficiency.

References:

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Anionic lipid nanoparticles preferentially targeting mRNA to hepatic RES *in vivo*

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Clinical translation of efficient and targeted drug delivery nanosystems is challenging, a major contribution to this is the insufficient knowledge of the factors influencing the nanoparticle circulation, uptake and the identification of key players in these mechanisms. Due to the dynamic nature of the involved mechanisms, an *in vivo* assessment is strictly required. We use the transparent and versatile embryonic zebrafish as an animal model to rapidly screen the behavior of nanoparticles *in vivo*. The ability to visualize fluorescent nanoparticles at cellular resolution in a living organism has given us an understanding of the fundamental behavior of nanoparticles.

Previously, we identified Stabilin-1 and Stabilin-2 as the main receptors responsible for the clearance and uptake of intravenously administered anionic nanoparticles [1,2]. Here, this knowledge was used to rationally design a lipid nanoparticle formulation able to preferentially target the hepatic reticuloendothelial system (RES). A single lipid was replaced within the lipid composition of the clinically approved Onpattro® to design srLNPs. This replacement changes the surface charge, from neutral to anionic, allowing the redirection of srLNPs to preferentially target hepatic RES. Upon injection in the zebrafish embryo, we showed the biodistribution of srLNPs and the subsequent preferential intracellular delivery of the carried mRNA. Remarkably, we show that srLNPs target scavenging endothelial cells is mediated by Stabilin-1 and Stabilin-2. Validation in mice confirmed the srLNP biodistribution, uptake, cytosolic delivery and protein expression of hepatic RES mice cells, opening up opportunities to treat liver diseases associated with RES.

This research demonstrates the zebrafish is a powerful model system to study mechanisms involved in nanoparticle-mediated drug delivery.

References

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A lipid nanoparticle RNA vaccine platform for induction of antigen-specific tolerance

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Aims

Globally, 5 to 10% of the population suffers from an auto-immune disease and generally are treated with immunosuppressive medication [1]. Induction of immune tolerance to specific antigens however, could potentially allow treatment without the trade-off of systemic immune suppression. Liver sinusoidal endothelial cells (LSECs) form the vascular lining of sinusoids in the liver and have previously been shown to mediate immune tolerance [2]. Our work has shown that lipid nanoparticles (LNPs) can specifically deliver mRNA coding for antigen to the zebrafish equivalent of LSECs [3]. Here we address whether LNPs specifically target LSECs and can induce antigen specific tolerance in mice.

Methods and Results

By priming C57BL/6 mice that received an adoptive transfer of ovalbumin (OVA)-specific CD4+ (OT-II cells) and CD8+T cells (OT-I cells), with LNPs that contain OVA-encoding RNA, we show that LNP administration substantially reduced (>10-fold) OT-I expansion upon an inflammatory challenge with OVA and Poly(I:C). Moreover, we observed a decrease in T-bet+ OT-I T cells and a significant increase in CD25+FoxP3+ OT- I cells. Interestingly, we did not observe any significant differences in expansion of OT-II cells, nor did we observe any changes in their activation or polarisation.

Conclusions

Overall, these results suggest that administration of OVA mRNA using LNPs leads to induction of tolerance mediated by antigen-specific CD8+ T cells. Currently, we are studying the application of LNPs that contain OVA-encoding RNA as a therapeutic vaccine in mice with established inflammation. Additional follow-up research includes studying the biodistribution of the LNPs in mice after intravenous administration. We aim to use this versatile vaccine platform to induce antigen-specific immune tolerance in auto-immune disease mouse models driven by a cytotoxic CD8+ T cell response to demonstrate the possible applications of this method in the clinic.

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