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Re: Reference Letter for Felix Romer

To whom it may concern,

Felix Romer worked on his MSc thesis entitled “Heterotypic Interactions Between Endothelial Cells and (Infected) Macrophages: Characterisation of Endothelial Cell Shape Alterations, Organisation, and Macrophage Adhesion” under my supervision until April 2025. Currently he is still working in lab as a HiWi until June 2025 on the same project. The project he works on is in a collaboration with a senior PhD student in my laboratory, Marie Muenkel, who also trained Felix Romer on how to work in a biosafety level 2 laboratory, perform tissue culture including with primary human cells, assemble and functionalize the vessel-on-chip device that was used partly for these studies, use our epifluorescence and our spinning disk confocal microscopes for imaging, and perform image processing.

Infectious diseases pose a global health concern being a significant cause of morbidity and mortality worldwide, which will be further exacerbated in the near future due to the emergence of antibiotic resistant strains. Yet, we still do not understand how all these pathogens can infect and spread throughout the human body. In particular, intracellular bacterial pathogens, like food-borne *Listeria monocytogenes* (LM), have developed a multitude of strategies of manipulating their host cells, ensuring they do not destroy host cell integrity since they rely on it, in order to spread systemically, without being exposed to the extracellular milieu, where they would be targeted by antibody-mediated immune responses. In my lab, Felix Romer was able to show that unlike naïve control endothelial cell (EC) monolayers, ECs in monolayer exposed to either uninfected or bacterial infected macrophages (MΦ) become increasingly polarized and collectively aligned in a common direction. To quantitate this feature of increased collective EC alignment in presence of MΦs, he used AFT (Alignment by Fourier Transform), a workflow that quantifies the alignment of fibrillar features in microscopy images via 2D Fast Fourier Transforms (FFT). These data will be included in a manuscript that is under preparation for submission and which is focused on the biomechanical alterations of ECs when interacting with bacterially-infected immune cells, and Felix Romer is a co-author in this work due to his contribution.

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Additionally, Felix Romer during his stay at my lab learned how to assemble and work with a vessel-on-chip device developed by the team of Prof. Peter Loskill with whom my lab is collaborating. Felix was able to seed ECs in monolayer and perfuse them for 24 h with low magnitude shear flow and then add MΦs for additional 2 h to assess how adhesion of MΦs to ECs depends on their infection status. Consistent with preliminary data of Marie Muenkel, he found a 2-fold increase in EC-MΦ adhesion when MΦs were infected as opposed to not. These results will also be included in the manuscript mentioned above, currently in preparation for publication. Finally, Felix performed experiments on ECs exposed for 24 h to flow (or not, controls) and residing on the vessel-on-chip device. He then fixed the samples and performed immunostaining followed by microscopy imaging and image processing, to characterize in a highly quantitative manner cell shape changes and organization alterations that ECs exposed to shear flow undergo as compared to those under static conditions. He found that the polarization of ECs increased during exposure to shear flow, with the cells aligning towards the direction of flow and increasing their collective alignment. This is a very interesting finding which opens the question of what would additionally happen if these ECs are now exposed to MΦs. This is a particularly important question since *in vivo*, in the vasculature, ECs are not under static conditions but are always subject to shear stresses due to blood flow that are well known to impact EC mechanobiology and important EC functions. But how those might contribute to enhancing or conversely obstructing infection dissemination is still unknown. Through his work Felix Romer, established a great framework for addressing such questions as well as a straightforward computational pipeline for analyzing the resulting data.

Felix Romer's thesis was very well written, including detailed protocols, with clear presentation of the results and discussion over how his results fit with what is already reported in the literature as well as what perspectives his findings open. His laboratory work was good, and he was well prepared for our one-on-one discussions presenting his research progress. During his stay in my lab, he learned to use various state-of-the-art instruments, and to analyze his data. His project was rather challenging because of the usage of the organ-on-chip device together with the performance of additional assays and involved often a high extent of prior planning, but Felix Romer was able to manage all that well. Felix is very well organized, hard-working, has strong communication and writing skills and a great team member to work with. Please do not hesitate to contact me should you need further information.

Yours sincerely,

Effie Bastounis

