# The role of pericytes during micro-infarction and angiophagy

# Introduction/Background

With an increasingly aging population, the burden of cognitive decline on society continues to increase while knowledge about what drives cognitive decline remains unresolved. Micro-stroke, also known as “silent infarct”, can be associated with brain damage and cognitive decline, caused by poor reperfusion after a small embolus occludes a capillary resulting in an ischemic event. These micro-infarcts are not treatable as diagnostic methods lack sensitivity and therapeutic agents are not established. Usually, silent brain infarcts are discovered in post-mortem tissue, limiting the possibility to study the onset and progression of silent brain infarcts' ischemic and hypoxic events. To resolve this problem, the microsphere animal model is used to mimic and study silent brain infarcts from an early onset. The microsphere animal model consists of the injection of polystyrene microparticles into the common carotid artery of a mouse or rat. The microparticles flow through the circle of Willis and the pial arteries

Figure 1: Schematic representation of out hypothesis that a pericyte can block the migration of a microsphere through the blood vessel with a smaller diameter than the microsphere. Additionally, we also hypothesize that during angiophagy pericyte pore formation enables the extravasation of the microsphere besides the endothelial pore formation.

at the brain surface into the penetrating arteries. The unpublished work of our group suggests that the 15-micrometer microparticles can move through the capillaries with approximately a diameter of 10 µm until the site where the microparticles occlude the vessel. We hypothesize that endothelial cells get activated by the increased mechanical forces from the microparticle and the blood pressure, resulting in vasodilation, structural remodeling of the endothelial cytoskeleton and extracellular fibers and leading to capillaries with a much larger diameter that enables the microparticle to move through the capillaries. However, these particles eventually do occlude the capillaries while we do not know what factor determines the location of the occlusion. These so-called micro-infarcts are causing small hypoxic and ischemic regions but the brain’s microvasculature is capable of resolving these hypoxic and ischemic regions over time by angiophagy. Angiophagy is the process of extravasation of occluding particles out of the vessel into the parenchyma. Although some research has described angiophagy, the exact mechanisms involved remain to be elucidated (1, 2, 3, 4, 5, 6).

Pericytes are spatially isolated contractile cells of the capillaries and are involved in a variety of functions, such as controlling the brain’s blood flow and regulation of immune cell entry(7, 8). It is already known that pericytes engulf emboli after angiophagy and that there are increased matrix metalloproteinases (MMPs) activity at the side of angiophagy(3, 9). However, it is not clear if the MMPs are secreted by the pericytes or the endothelium and how the pericytes are involved in the onset and progression of angiophagy. Additionally, due to the disruption of the blood flow and hypoxia downstream of the micro-infarction, it might be possible that the pericytes are constricted(7). Constriction of the pericytes might be a barrier for the microparticles when slowly flowing through the capillaries. Overall, it is not completely clear how pericytes are involved in the angiophagy of micro-emboli.

In this project, we will focus on the function of pericytes during micro-infarction and angiophagy. We hypothesize that pericytes can form a barrier for the migration of microspheres that flow through the blood vessels (fig.1). Secondly, we hypothesize that pericytes are actively involved in the process of angiophagy and that it might look similar to leukocyte extravasation (fig.1). We will address this by looking at pericyte coverage and morphology on the capillaries near microparticles. Additionally, we will investigate if there is pericyte constriction around the microspheres during angiophagy to determine if pericyte constriction plays a role in the lodging of the microspheres . Finally, we will look at the expression levels of multiple endothelium and pericyte markers to determine their involvement before and during angiophagy.

# Research questions

1. Do pericytes play a role in determining the site of micro-embolization?
2. Does the morphology of pericytes change during angiophagy?
3. Do the expression levels of endothelial, pericyte, and extracellular matrix proteins change during angiophagy?

# Biomedical/clinical background

Increasing our understanding of vascular plasticity and its response to occluding particles in the bloodstream is crucial for the development of new therapeutic approaches for stroke.

# Research material, plan and data collection

Mice used for this study are perfused with 10 µm microspheres via the common carotid artery (CCA) and sacrificed 1, 7 or 21 days post-surgery. In this project we will use the fixed tissues of these mice to stain the vasculature and pericytes to study the role of the pericytes during microsphere migration through the vasculature and during angiophagy. As a start, the student will be involved in processing the brain and cutting of the free-floating tissues. Next, we will examine the tissue quality and decide if the tissues can be included in the studies.

Immunohistochemistry staining’s for this study will include vessel and pericyte markers CD31 and PDGFR-β. We will include 6 mice for each time point (total n=18). We will image the all microspheres present in the tissues via confocal microscopy until we obtained minimally 5 microspheres in straight vessels. Out of precious experience we know that it is highly likely that we will also encounter microspheres in bifurcations and therefore the total imaged microspheres per mice might be higher.

What follows is the analysis of a series of different parameters. The 1 day post-surgery tissues will be used to answer the first research question. We will examine the pericyte location in relation to the microsphere and determine the vessel diameters near the pericyte and microsphere.   
Post-mortem tissues from day 7 and 21 will be used to determine the role of pericytes during the extravasation of the microspheres. We will look at the morphology of pericytes and try to classify what kind of pericyte is involved. Finally, we will look at expression levels of different protein levels at the sight of angiophagy to get a better understaning in the mechanism that are involved during the process.

# Data collection and analyses

Immunohistochemistry, confocal microscopy, and image analysis using imageJ-vasometrics to measure vessel diameter, NIS-elements for expression analysis.

# Literature

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