Force sensing and transmission in human induced pluripotent stem-cell-derived pericytes

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General Summary and Discussion

Pericytes, the mural cells of blood microvessels, have been described for the first time in 1873 by the French scientist Charles-Marie Benjamin Rouget as a subset of contractile cells surrounding microvessels [1]. In 1923 Zimmerman introduced a new term "pericytes" to connect to their close location to the endothelial cells that form vessel tubes [2]. Pericytes cover the majority of all capillaries and over the years have emerged as important regulators of vascular morphogenesis and function. Despite extensive studies, there are remaining important unsolved questions related to the mechanobiology of pericytes [3] with one of the most intriguing parts of them – the control of the vascular blood flow [4–8].

Currently, the mechanical behaviour of pericytes, although of significant importance to their biological function, can only be inferred from (post mortem) *in vivo* imaging. The main reason for this lack of accessibility is the difficulty of generating homogeneous cell culture of primary mid-capillary pericytes, as they show differences in phenotype and expression of proteins depending on their location on the capillary tree and don't have unique markers [2, 9, 10]. Additionally, "true" or "approved" pericytes are located on mid-capillaries that show the most clear differences from smooth muscle cells [9, 11]. Pericytes located on pre- or post-capillaries have been defined as "transitional" pericytes [2]. Thus, generating a cell culture of "true", mid-capillary, primary pericytes that don't show expression of α-smooth muscle actin (α-SMA) and support angiogenesis is nearly impossible. As a confirmation, there are no such commercially available primary pericytes present. *In vivo* experiments lack the level of control of experimental conditions and don't support direct cell force measurements in contrast to *in vitro* studies.

In this thesis an attempt was made to push boundaries and develop approaches to study pericyte mechanobiology *in vitro*. First, by us-

ing human induced pluripotent stem cell-derived pericytes that resemble "true" pericytes located on mid-capillaries, characterized by the lack of α-smooth muscle actin and supporting angiogenesis [12, 13]. Pericytes have a complicated biochemical and spatial organization of extracellular matrix proteins around them on the capillaries, combined with changing stiffness, accompanying changes in the blood pressure. These factors generally have implications for cell behavior and pericytes are not an exception [14–16]. It is important to combine all these factors in order to accurately reproduce the cell microenvironment *in vitro*. In **chapter 2** the existing knowledge about the structure and properties of the pericyte extracellular matrix have been utilized to identify most appropriate methods to re-create the mechanical microenvironment of pericytes *in vitro* that open new opportunities to study their mechanobiology.

Pericytes are embedded within the capillary basement membrane made of the three main components – collagen IV, laminin-411/511 and fibronectin that are used for attachment of cells in the capillary wall [17–20]. Recent findings revealed that laminin and type IV collagen are not organized into one homogeneous network, but form two layers, allowing fibroblasts to interact with collagen on one side and endothelial cells to interact with laminin on the other side of the basement membrane [21]. Pericytes turned out to be located "under" the collagen layer close to endothelial cells with a thin laminin rich layer in the pericyte-endothelial cell interstitia. Moreover, by electron microscopy it was found that this laminin layer contains small deposits of fibronectin [17, 20, 22]. While endothelial cells attach mainly to laminin, for pericytes this is less well understood. The *in vitro* model of the basement membrane structure in the pericyte-endothelial cell interstitia of the mid-capillary region that I developed allowed to show in **chapter 3** that pericytes prefer fibronectin over the laminin, pointing to a potential role of these fibronectin deposits as main anchoring points for mechanical attachment of pericytes to capillaries. This role was originally proposed by Courtoy in 1983 and now I was able to confirm in an *in vitro* experiment, using the recent advances in the extracellular matrix modeling approaches.

I showed that pericytes respond to the variation in fibronectin-patterned substrate stiffness with changes in force application, spreading, and cell-matrix adhesions size in a not linear manner as, for example, human or mouse fibroblasts, where cellular traction forces together with spreading and cell matrix adhesions size gradually increase with increasing

substrate stiffness [23–25]. Pericytes show optimal spreading and cell-matrix adhesions size on intermediate substrate stiffness and suppressed on both soft and stiff substrates. The forces applied by pericytes do not follow the same trend and are vice versa lower on intermediate substrate stiffness and higher on soft and stiff. The stiffness range supporting optimal pericyte spreading appeared to be close to that for endothelial cells and smooth muscle cells determined by atomic-force microscopy [26, 27], indicating that this stiffness range represents a response in a physiologically relevant stiffness regime. Behavior of pericytes observed in this study gives an insight on the way pericytes distinguish deviations of the microvessel stiffness from the normal tissue and react by increasing contractile forces to provide a mechanical support for microvessel walls, preventing excessive dilation.

As aforementioned, depending on the location along the microvascular tree pericytes have been divided into three subgroups: pre-capillary, mid-capillary and post-capillary pericytes. Mid-capillary pericytes completely lack α-smooth muscle actin, while pre- and post-capillary pericytes show a gradient in the α-smooth muscle actin expression levels from low, next to mid-capillaries, to high closer to arterioles and venules where smooth muscle cells come in place [5, 9, 10]. *In vivo* and *in vitro* studies on pericytes and other cell types show that α-SMA expression is largely affected by soluble factors, but also can be attenuated by mechanical stimuli like substrate stiffness and extracellular matrix. Taking into account that the change of the capillary order is accompanied by the change in it's diameter, inside blood pressure, basement membrane thickness and protein composition, pericytes experience different mechanical signals on different parts of the microvascular tree and this may have an influence on α-SMA protein expression that leads to contractility. Earlier findings already pointed on a special role of extracellular matrix mechanical properties in the α-SMA regulation in myofibroblasts [28] as well as in mesenchymal stem cells [29]. Whether and how do these factors combine to condition α-SMA expression gradient in pericytes in the resting vasculature remains unclear.

In **chapter 4** our *in vitro* approach to study pericyte mechanobiology allowed to investigate whether such parameters like vessel diameter, basement membrane composition and stiffness can have an effect on the α-SMA recruitment to stress fibers in pericytes. An image analysis approach was utilized to obtain uncompromised data on the α-SMA fiber

formation with a single cell resolution. It was observed that pericytes seeded on fibronectin dots surrounded by laminin, showed a lower percentage of α-SMA recruitment to stress fibers than pericytes seeded on a monolayer of fibronectin. The first pattern resembles protein organization in the mid-capillary pericyte-endothelial cell interstitia and the second is more common for arteriole and venule regions of a microvasculature tree. This data suggests an inhibitory effect from the fibronectin organized in patches in the capillary basement membrane on the α-SMA recruitment to the F-actin cytoskeleton of pericytes. Likewise, pericytes showed low to no additional correlation of the α-SMA recruitment with the stiffness or vessel diameter in the presence of fibronectin organized in a dotted pattern. In contrast, human smooth muscle cells maintained the ability to form α-SMA fibers on such a patten and responded to the deviating stiffness and available area for spreading with different α-SMA recruitment rates, yet higher then in pericytes. This data demonstrates that after full maturation of pericytes into smooth muscle cells they loose the ability to adjust α-SMA expression levels in response to the fibronectin arrangement in the basement membrane and become more dependent instead on vessel stiffness and diameter. These findings may further help to unveil processes behind maintaining α-SMA expression gradient in pericytes, and can be used to keep them from obtaining contractile phenotype in cell culture.

The contractility of pericytes, despite some contradictory results, was reported to be involved in regulation of the cerebral blood flow and promote brain damage in ischemia in mice *in vivo* [4, 5, 8]. Nevertheless, there is no direct evidence in terms of direct mechanical measurements. In **chapter 5** a new approach was designed and characterized that allows to constantly monitor cellular forces during a rapid exchange of cell culture media to expose cells to different environmental conditions such as: the temperature, the composition of the cell culture medium, and the oxygen concentration. This approach permits the study of the behaviour of pericytes during hypoxia and ischemia. It also can be applied to any other cells for which the environmental conditions need to be changed, and where responses in cell force application and morphology are questioned.

Taken together, the approaches developed in this thesis to study pericyte behaviour *in vitro* and the obtained results show novel opportunities to investigate pericyte mechanobiology and provide insight, better

understanding and proof to processes that were impossible in *in vivo* experiments.

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