

# Role of Pericytes in Neurovascular Unit and Stroke

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## Abbreviations

ACA	Anterior cerebral artery
BBB	Blood–brain barrier
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CNS	Central nervous system
MCA	Middle cerebral artery
NVU	Neurovascular unit
PA	Penetrating arteries

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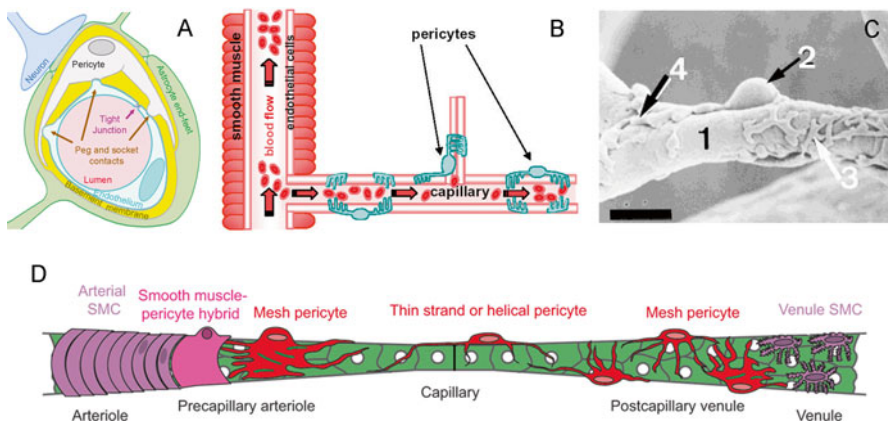
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PDGFR $\beta$	Platelet-derived growth factor receptor- $\beta$
PDGF $\beta$	Platelet-derived growth factor- $\beta$
RBCs	Erythrocytes = red blood cells
SMCs	Smooth muscle cells
tPA	Tissue plasminogen activator
$\alpha$ SMA	Smooth muscle $\alpha$ -actin

## 1 Neurovascular Unit and Stroke

The neurovascular unit (NVU) is composed of the endothelia, pericytes, basal membrane ensheathing them, astrocyte end-feet around the vessels and, neurons innervating the intraparenchymal vasculature (Fig. 1a). It plays an integrating role in matching the metabolic demand with the blood flow in addition to regulating the development and maintenance of the blood–brain barrier (BBB) [1–5], leukocyte trafficking across the BBB [6–9], and angiogenesis [10–12]. Ischemic injury to the NVU unfavorably impacts the stroke-induced damage and brain edema by



**Fig. 1** Neurovascular unit and pericytes. (a) The neurovascular unit is composed of the endothelia and tight junctions between them, pericytes, the basal lamina ensheathing endothelia and pericytes, and astrocyte end-feet surrounding the microvessel. Note the peg and socket type contacts between endothelia and pericytes. (b) Potential blood flow control sites in cerebral vasculature: smooth muscles on arterioles and pericytes on capillaries (Reproduced from Peppiatt et al., 2006 with permission). (c) Scanning micrograph of a vascular cast of a cortical capillary (1) with a pericyte-like structure (2) having primary and secondary processes (3) distributed around the vascular cast and the capillary branching points (4). Scale bar = 11.5  $\mu$ m. (Reproduced from Rodríguez-Baeza et al., 1998 with permission). (d) Pericyte processes are highly varied with shapes ranging from thin singular strands that run parallel to the microvasculature to more complex mesh processes that enwrap the entire vessel lumen. Pericytes located closer to the arteriolar end of the microcirculation exhibit more circular processes that may be essential to their contractile function (Reproduced from Hartmann et al., 2015 with permission)

disrupting microvascular blood flow and BBB integrity, whereas ischemia-triggered signaling in the NVU of vasculature within the peri-infarct area positively impacts stroke outcome by promoting post-stroke angiogenesis and neurogenesis [13, 14].

## 2 Pericytes

First described by Rouget in 1873 [15], pericytes were later named by Zimmerman in 1923 based on their prominent location at the abluminal wall of microvessels [16]. However, their importance within the NVU has not been recognized until recently. Pericytes communicate with other cells of the NVU and contribute to the control of several microcirculatory functions such as neurovascular coupling, maintenance of the BBB and basal lamina, regulation of the angiogenesis, immune cell entry to the central nervous system (CNS) and scar formation [3, 11, 17–21]. They also function as pluripotent stem cells after injury to the NVU [22]. Although pericytes are also present on peripheral microvessels, the density of pericytes on microvessels is highest in the CNS and retina in accordance with their role in focal regulation of the microcirculatory blood flow and maintenance of the blood–brain/retina barrier [2, 21, 23, 24].

Pericytes are located on pre-capillary arterioles, capillaries, and post-capillary venules [2, 25, 26] (Fig. 1b–d). Pericytes are present on straight parts as well as branching points of the capillaries; 56 % of the pericyte somas are located at a capillary junction [27]. Unlike smooth muscle cells (SMCs), pericytes are embedded within two layers of basement membrane [24]. They form peg and socket type contacts and gap junctions with the endothelia through the basement membrane, facilitating the communication between pericytes and endothelia [28, 29] (Fig. 1a). Adjoining membranes of the neighboring pericytes are interconnected with gap junctions, which probably serve as a conduit for transmitting messages along the microvascular wall [19, 30]. Pericytes extend processes along and around the microvessels, which cover 30–90 % of the microvessel wall in the CNS [4, 23, 31, 32]. Processes are more circumferential at the arteriole side of the microvascular bed and at branching points, more longitudinal in the middle of the capillary bed, and have a stellate morphology at the venule end of the microcirculation (Fig. 1d). Junctional pericytes extend processes at least a cell width away from the junction [27]. It has long been recognized that pericyte morphology varies along the course of microvasculature, presumably to accommodate differing functions [25, 27, 33]. Not only their morphology, but also their protein expression varies [2, 34]. Several transitional forms are observed along the vascular bed at various developmental stages or after pathological stimuli [2, 25, 26, 35]. The transition from smooth muscle  $\alpha$ -actin ( $\alpha$ SMA) expressing SMCs to pericytes is also not sharp. Smooth muscle-pericyte “hybrid” cells precede the prearteriolar pericytes having mesh-like circular processes [26, 27]. Pericytes that give out more circumferential processes express more  $\alpha$ SMA, when assessed either with immunohistochemistry of brain sections *ex vivo* [27, 34] or in mice cortex expressing reporter dyes under control of the

$\alpha$ SMA promoter in vivo [33]. It should be noted that immunohistochemistry directly detects the  $\alpha$ SMA protein (mainly in the cytoplasm of the soma and processes), whereas reporter dyes expressed under the control of  $\alpha$ SMA promoter are membrane-bound, therefore, basically label the pericyte membrane. Mid-capillary pericytes do also express  $\alpha$ SMA [36]. However, the detection of the small pool of  $\alpha$ SMA in their relatively short processes by immunohistochemistry requires rapid fixation before  $\alpha$ SMA depolymerization, whereas low level of  $\alpha$ SMA expression may be difficult to visualize due to dispersion of the limited amount of reporter fluorescent protein expressed over the large surface area of the pericyte membrane.

Pericytes can be identified by a number of proteins that they express. Unfortunately, the antibodies against to majority of them either detect only a subpopulation of pericytes or do not have the desired specificity to unambiguously distinguish pericytes from other cell types [2, 25]. Recent studies with transgenic mice expressing fluorescent reporter proteins under the control of the growth factor platelet-derived growth factor receptor- $\beta$  (PDGFR $\beta$ ) and of the proteoglycan NG2, suggest that PDGFR $\beta$  detects a larger population of pericytes compared to NG2 and, interestingly, CD13 immunohistochemistry is reportedly to match the performance of fluorescent reporters expressed under the control of PDGFR $\beta$  promoter [27, 33, 37].

## **2.1 Regulation of Microcirculatory Blood Flow and BBB by Pericytes**

Pericytes are considered contractile cells since their discovery [15, 16]. However, this view was challenged in the past few decades based on the premise that the blood flow increase evoked by neuronal activity was mediated by relaxation of SMCs around arterioles as well as by failure of detection of  $\alpha$ SMA in capillary pericytes in some studies. As reviewed in detail by Dfáz-Flores et al. [12], the pericyte contractility is supported by several lines of evidence including their characteristic morphology with processes that envelop the microvessels as well as ultrastructural and immunohistochemical demonstration of contractile proteins [24, 34, 36, 38–45] in addition to the presence of receptors for vasoactive mediators on their surface [19, 30, 46]. In vitro studies on cerebellar, cerebral, and retinal slices or on isolated microvessels and in vivo studies have clearly disclosed that pericytes contract or dilate in response to vasoactive mediators applied [30, 46, 47]. A recent in vivo study showed that cortical capillaries dilated before arterioles during sensory stimulation, supporting the view that microvascular blood flow in the CNS is regulated by pericytes in response to the very focal demand originating from a small group of nearby cells as a final step of flow regulation after the arterioles, which serve a larger cohort of cells [18]. This flow regulation with fine spatial resolution may be essential for tissues with high functional specialization such as the brain and retina. However, it should be noted that all microvascular pericytes are not contractile and proportion of the contractile ones may vary with the tissue, species, and developmental stage as well as along the arteriovenous axis as noted above [20, 33, 48].

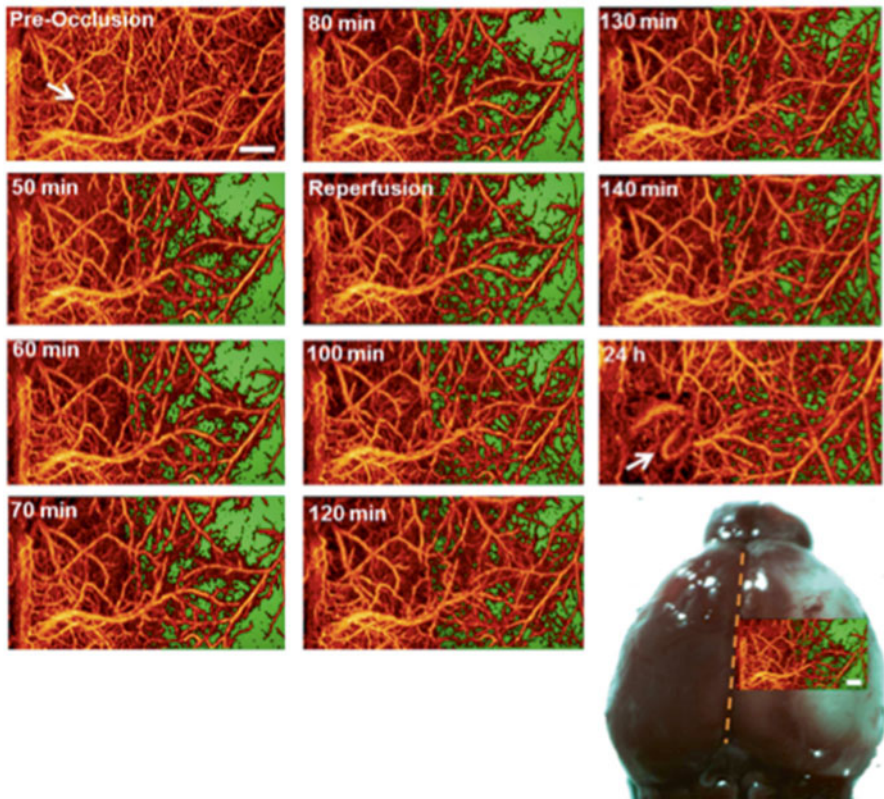
It has also been shown that a close interaction between the endothelia and pericytes as well as astrocytes is required for development and functioning of the NVU and BBB [4, 49]. Pericytes promote the formation of tight junctions and inhibit transendothelial vesicular transport [4, 49, 50]. The number of pericytes per endothelial cell and the surface area of the vascular wall covered by pericytes determine the relative permeability of capillaries. Accordingly, pericyte dysfunction or deficiency causes increased BBB permeability [2, 4, 21, 49].

## ***2.2 Pericytes Are Vulnerable to Ischemic Injury***

The pericyte contractility is regulated by intracellular  $\text{Ca}^{2+}$  concentration as in vascular SMCs surrounding the upstream vessels [19, 46, 51]. Accordingly, these highly dynamic cells bear the risk of  $\text{Ca}^{2+}$  overload when they are unable to maintain low intracellular  $\text{Ca}^{2+}$  concentrations. The energy insufficiency is a well-characterized cause of loss of the intracellular  $\text{Ca}^{2+}$  equilibrium, which relies on several energy demanding processes. In addition, factors such as reactive oxygen species (ROS) may also contribute to an uncontrolled rise in intracellular  $\text{Ca}^{2+}$  [52, 53]. Pericytes have large elongated mitochondria, which follow the central core of the pericyte longitudinally, and may be a significant source of ROS under pathological conditions [31]. NADPH oxidase, a major superoxide-producing enzyme is highly expressed in brain pericytes [54, 55]. Indeed, ROS has been shown to cause a sustained increase in  $\text{Ca}^{2+}$  in cultured human brain microvascular pericytes [52, 53]. Reactive oxygen and nitrogen species, especially peroxynitrite, have been reported to induce pericyte contraction during focal ischemia/reperfusion in the intact mouse brain [56]. In addition to unregulated  $\text{Ca}^{2+}$  rise, several other processes such as ATP and thromboxane A<sub>2</sub> released from the ischemic brain or platelets, which are potent constrictors of pericytes, may also contribute to pericyte contraction [48].

## **3 Changes in Pial and Penetrating Arteries Shortly After Stroke**

On occlusion of the middle cerebral artery (MCA), collaterals between the anterior cerebral artery (ACA) and MCA are opened, large arterial branches but especially surface pial network and penetrating arteries (PA) dilate while flow directions are reorganized to sustain the flux rates in PAs as high as possible [57–59]. While these compensatory changes can preserve cell viability at the periphery of the MCA area, creating an opportunity for recovery if MCA recanalization can be attained within a couple of hours, the severe decrease in blood flow velocity, volume, and distal capillary perfusion in the core ischemic area makes infarction unavoidable [58, 60–62] (Fig. 2). In vivo imaging of cerebral circulation in intact mice under anesthesia has unequivocally illustrated that the PAs, especially those with smaller luminal



**Fig. 2** Incomplete microcirculatory reflow after recanalization. Dynamic imaging of cortical blood flow using optical microangiography during 90-min proximal MCA occlusion followed by recanalization illustrates the lack of microcirculatory blood flow in the MCA territory (the *green area*) during occlusion and its partial recovery after recanalization (incomplete microcirculatory reperfusion) in the mouse. Consecutive images are shown at 10-min intervals. Image size is  $2.2 \times 4.4 \text{ mm}^2$ . The image in the *lower right* is the optical microangiography image taken at 50 min overlaid on the 24 h infarct analysis by histological staining as the area of pallor. Scale bar =  $500 \mu\text{m}$  (Reproduced from Dziennis et al., 2015 with permission)

diameter dilated to compensate for the low perfusion pressure [58, 61]. The magnitude of dilation decreased with the distance from the pial arteriolo-arteriolar anastomoses with sufficient collateral flow and was replaced by constriction in areas further away [58]. Majority of these changes are reversible if recanalization is achieved within a short time. However, when perfusion deficit is prolonged some of these changes are not reversible and may negatively impact the recovery after stroke. For example, in the mouse brain, part of the microcirculatory flow cannot be reinstated after MCA occlusion lasting more than an hour despite complete reopening of the MCA [33, 56].

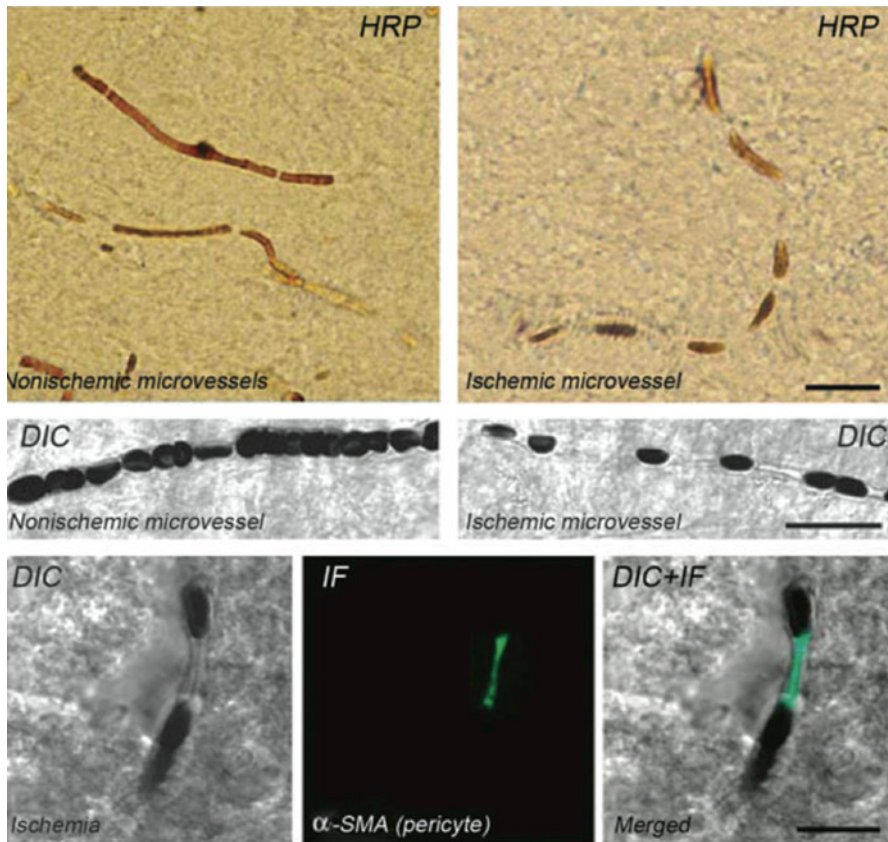


## 4 Incomplete Microcirculatory Reflow After Recanalization

An impaired tissue reperfusion due to microvascular constrictions (no-reflow phenomenon) was first noted after global and focal cerebral ischemia more than half a century ago [63, 64]. The emergence of “no-reflow” depends on the duration and severity of ischemia as well as the brain region studied although these variables have not been systematically compared in the setting of focal cerebral ischemia. In the mouse, MCA occlusion induces nodal microvascular constrictions that generally do not recover after recanalization starting 1 h after ischemia and affecting more than half of the microvessels within 2 h [33, 56] (Fig. 2). Narrowed microvessel lumina are filled with entrapped erythrocytes (RBCs), leukocytes, and fibrin-platelet deposits [65–69]. RBCs are the predominant cell types in aggregates as they are the most prevalent cells in circulation. In addition to the constricted segments observed at the arteriolar end of microcirculation and capillaries, leukocytes adhered to post-capillary venules for entering to the parenchyma also induce luminal aggregates together with fibrin and platelets [65, 69–71].

Experimental data strongly suggest that incomplete restoration of the microcirculatory blood flow negatively impacts tissue recovery even if reopening of the occluded artery is achieved when there is still salvageable penumbral tissue. Pharmacological agents and genetic manipulations reducing microvascular clogging by inhibiting leukocyte adherence, platelet activation or fibrin–platelet interactions have been shown to restore microcirculation and improve stroke outcome in animal models [65, 72–75]. Importantly, neuroprotection obtained with some BBB-impermeable agents strongly support the idea that restoring microvascular patency can improve stroke outcome independently of parenchymal mechanisms [56, 76]. Consequently, restitution of the microcirculatory reperfusion emerges as an exciting target to improve the success rate of recanalization therapies.

In the past, microvessel constrictions were thought to be caused by swollen astrocyte end-feet encircling microvessels [66, 67]. Recently, pericytes on microvessels were proposed to play an important role in incomplete microcirculatory reperfusion because they contracted during ischemia and remained contracted despite reopening of the occluded artery [18, 56, 77] (Fig. 3). Although it has been claimed that  $\alpha$ SMA expressing microvascular cells with contractile capability should be defined as SMCs [33], the mural cells with a bump-on-a-log morphology located on the abluminal wall of microvessels downstream to arterioles, including their transitional forms to SMCs, are named as pericytes since their original description by Zimmerman [16, 78]. The fact that pericytes are a heterogeneous group of cells sharing some transitional features with SMCs and that some but not all express  $\alpha$ SMA have always been a matter of confusion and a source of debate. Nomenclature disagreements notwithstanding the important point for the stroke pathophysiology is that contractile cells on brain microvessels impede reperfusion after ischemia and unfavorably impact the outcome of recanalization. It should be noted that even small decreases in capillary radius caused by subtle pericyte contractions can lead to erythrocyte entrapments because capillary luminal size hardly allows passage of



**Fig. 3** Ischemia causes persistent pericyte contraction, which is not restored after complete recanalization of the occluded artery. Mice were subjected to 2 h of proximal MCA occlusion and intravenously injected with horseradish peroxidase (HRP) before decapitation, 6 h after reopening of the MCA. HRP-filled microvessels exhibited sausage-like segmental constrictions in ischemic areas on brain sections (*upper row*). The differential interference contrast (DIC) microscopy images illustrate frequent interruptions in the erythrocyte column in an ischemic capillary contrary to a continuous row of erythrocytes flowing through an intact capillary (*middle row*). The constricted segments colocalized with  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) immunoreactive pericytes (*bottom row*). IF denotes immunofluorescence. Scale bar for *upper* and *middle row*, 20  $\mu$ m; *bottom row* 10  $\mu$ m (Reproduced from Yemisci et al., 2009 with permission)

RBCs [19, 56] (Fig. 3 middle row). Entrapped erythrocytes trigger platelet and fibrin aggregation by impeding passage of blood cells [69, 79]. The failure of erythrocyte circulation within some of the microvessels and increased heterogeneity of RBC transit times through patent capillaries (due to varying degrees of capillary resistances) can catastrophically reduce  $O_2$  delivery to the tissue struggling to recover from ischemia-induced perturbations [80]. Since the plasma flow in constricted capillaries is relatively less restricted compared to RBC flux, glucose supply to some parts of the tissue may exceed  $O_2$  supply and stimulate anaerobic glycolysis,

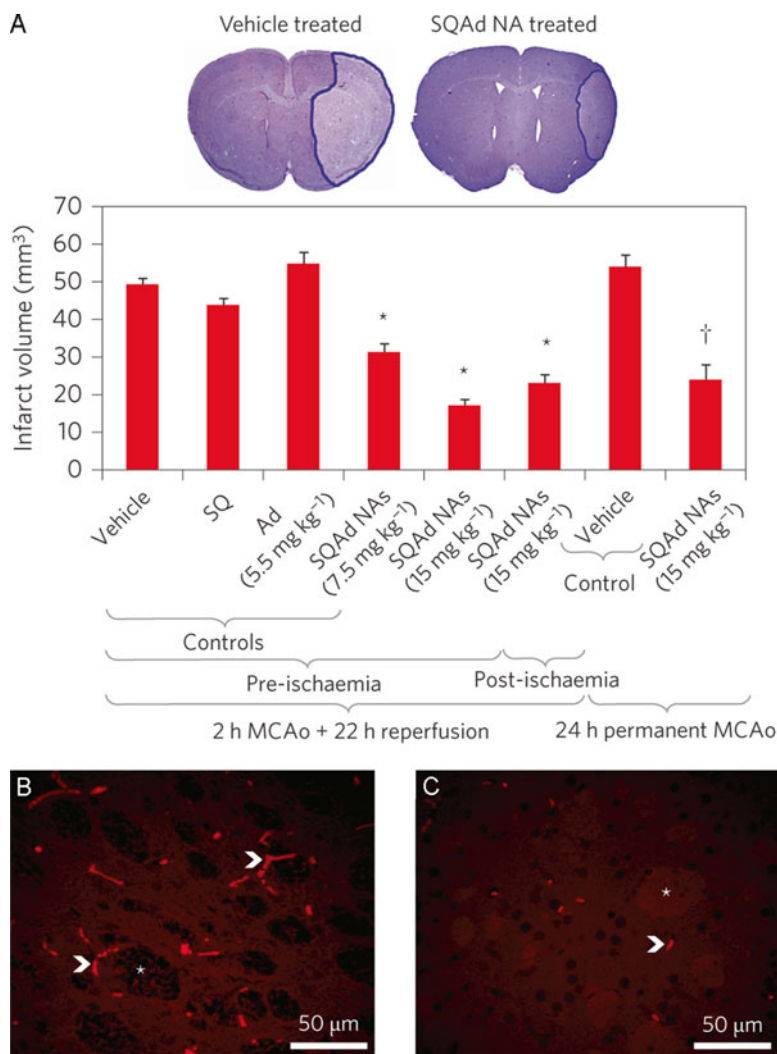


hence, lactic acidosis ([56, 77], please also see supplementary movies 5–7 in [33]). Therefore, ischemia-induced pericyte contractions emerge as a viable target for restoring impaired microcirculatory reperfusion. Indeed, sustained release of adenosine within circulation from nano-assemblies (NA) (adenosine itself has only a few minutes of plasma residence time) has recently been shown to reduce ischemia-induced erythrocyte entrapments and improve microcirculatory reflow by relaxing contracted pericytes after 2 h of MCA occlusion [76] (Fig. 4). Unlike adenosine infusion or synthetic adenosine agonists, slow release from squalenoyl-adenosine NAs did not cause cardiotoxicity or hypotension in the mouse model used.

Since pericytes also play an important role in maintenance of the BBB integrity [4, 21, 49], the ischemia/reperfusion-induced pericyte dysfunction may contribute to BBB leakiness as well. This can be further aggravated by the death of capillary pericytes within 24 h after MCA occlusion as shown in mice and rats [18, 81]. Increased BBB permeability predisposes to intraparenchymal hemorrhage and brain swelling in about 6% of patients receiving i.v. tissue plasminogen activator (tPA) [82]. Diabetic patients are more prone to hemorrhage perhaps due to dysfunctional microvascular pericytes, a well-known cause of diabetic retinopathy [83–85]. Interestingly, pericyte loss is increasingly reported for conditions that are risk factors for stroke, such as ageing, hypertension, and diabetes, the impact of which on stroke outcome needs to be clarified with future research [86]. Among many complex mechanisms, overproduction of oxygen and nitrogen radicals on the microvascular wall appears to contribute to both BBB leakiness and incomplete reflow during cerebral ischemia/reperfusion [56, 87]. Altogether, these findings bring about the exciting possibility that effective suppression of oxidative/nitrative stress during reopening of the occluded artery may improve the outcome of recanalization therapies by promoting microcirculatory reperfusion as well as by preventing hemorrhagic conversion and vasogenic edema [87]. Despite failure of an antioxidant agent in clinical trials, the experimental evidence still warrants pursuit of this goal [88, 89].

## **5 Clinical Evidence for “No-Reflow” After Recanalization Therapies for Stroke**

A short therapeutic time window limits the use of recanalization therapies for majority of stroke patients [90, 91]. This brief therapeutic time window is attributed to rapid loss of neuronal viability in the ischemic penumbra [82, 92]. However, increasing clinical evidence suggests that an incomplete reperfusion plays a critical role in determining tissue survival after successful recanalization [93, 94]. Several recent imaging studies serially analyzing recanalization and reperfusion in ischemic stroke patients report that, on average, 26% of recanalized patients with thrombolytics do not show reperfusion [95]. This incomplete reperfusion is observed after pharmacological (intravenous or intraarterial) as well as interventional recanalization [93, 96–98]. Clinical trials have repeatedly demonstrated that a good outcome was better correlated with reperfusion than recanalization in stroke patients treated



**Fig. 4** Systemic administration of squalenoyladenine (SQAd) nano-assemblies (NAs) provides significant neuroprotection in a mouse model of focal cerebral ischaemia. **(A)** Infarct areas in control and treated mice subjected to transient (2 h MCAo and 22 h reperfusion) and permanent (24 h MCAo) focal cerebral ischemia were identified by reduced Nissl staining under a light microscope (magnification  $\times 10$ , insets) (data are presented as mean (mm<sup>3</sup>)  $\pm$  S.D.,  $N=6$  animals per group;  $\dagger$  and  $*$  indicate  $P<0.05$  compared to respective controls). Intravenous administration of 7.5 or 15 mg kg<sup>-1</sup> SQAd NAs just before ischemia or 2 h post-ischemia significantly decreased the infarct volume compared with control groups that received vehicle (dextrose 5%), adenosine-unconjugated SQ NAs (9.45 mg kg<sup>-1</sup>) or free adenosine equivalent to the amount in NAs (5.5 mg kg<sup>-1</sup>). A significant therapeutic effect was also observed when SQAd NAs were administered 2 h post-ischemia in the permanent MCAo model. **(B, C)** In untreated mice, capillaries in the ischemic brain were filled with trapped erythrocytes, whose hemoglobin was rendered fluorescent by treating brain sections with NaBH<sub>4</sub> **(B, red, arrowheads)** 6 h after reopening of the MCA following 2 h of occlusion, whereas the majority of capillaries were not clogged in SQAd NAs-treated mice **(C)**

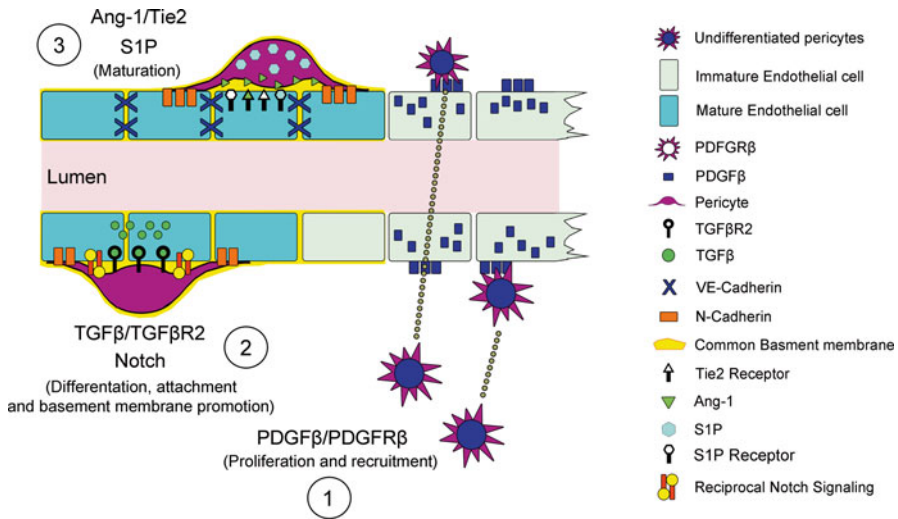
with tPA or interventional methods [93, 94, 96, 97, 99, 100]. Recent imaging studies show that increased capillary transit time heterogeneity (a measure of incomplete/impaired reperfusion) is a good predictor of the tissue destined to infarct [101].

## 6 Role of Pericytes in CADASIL

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the *NOTCH3* gene [102]. The protein encoded by the *NOTCH3* gene is expressed in pericytes as well as vascular SMCs. Recent studies in Notch3 transgenic mice expressing one of the human mutations have disclosed that Notch3 aggregated around microvascular pericytes, leading to pericyte loss or reduced coverage of capillaries by pericyte processes [103, 104]. These changes were associated with a leaky BBB, reduction in communication with endothelial cells and neurovascular dysfunction. Confirming the clinical significance of these findings, pericyte loss was also observed in skin and muscle biopsies of CADASIL patients [105].

## 7 Post-stroke Angiogenesis and Pericytes

Pericytes are essential especially for the early phase of neovascularization (angiogenic sprouting) [10, 11] (Fig. 5). Pericytes and endothelial cells communicate with each other for regulation of angiogenesis [10–12]. Platelet-derived growth factor- $\beta$  (PDGF $\beta$ ), transforming growth factor- $\beta$ , notch, angiopoietin, and sphingosine-1-phosphate signaling mediate this crosstalk [2, 106]. Increasing evidence suggests that pericytes play an important role in post-stroke angiogenesis as well [107–111]. Typically, endothelial cells start to proliferate and give off vessel sprouts 12–24 h after brain ischemia, leading to formation of new vessels in the peri-infarct region 3 days after ischemic injury [107, 112, 113]. Following a similar time course, the PDGFR $\beta$  expression is upregulated in pericytes, which increase in number and start migrating from the microvessel wall to the newly formed vessel sprouts to foster their maturation after ischemic injury [114–117]. Renner et al. found that PDGFR $\beta$  increased in pericytes 48 h after permanent ischemia [117]. Similarly, NG2+ or PDGFR $\beta$ + pericytes reportedly increase in peri-infarct areas 1–3 weeks after transient MCA occlusion [81, 118]. A proportion of locally proliferating pericytes give rise to microglial cells [119]. Interestingly, chronic administration of cilostazol, an antiplatelet drug, has been claimed to promote pericyte proliferation, which might decrease the final infarct size by promoting new vessel formation after naturally occurring stroke in spontaneously hypertensive rats [120]. Corroborating these studies, conditional knockout of PDGF $\beta$ /PDGFR $\beta$  signaling in adult mice that have normally developed brain vasculature, led to larger infarcts than controls when subjected to focal cerebral ischemia [121]. Similarly, Zechariah et al. showed that



**Fig. 5** Role of pericytes in angiogenesis. The interaction between PDGFβ secreted by the endothelium and its receptor localized on pericytes (PDGFRβ) is essential for recruitment of undifferentiated mesenchymal cells/pericytes to newly formed vessels. Once pericytes are at the vascular wall, reciprocal Notch signaling between the endothelia and pericytes as well as interactions between TGFβ secreted by endothelial cells and its receptor TGFβR2 located at pericytes differentiate mural cells and attach them to the newly formed vessels. The TGFβ/TGFβR2 interaction also promotes formation of the common basement membrane and stabilizes newly formed vessels by inhibiting endothelial proliferation. Ang-1, which is secreted by pericytes, activates its endothelial receptor Tie2 and promotes blood–brain barrier formation. Finally, S1P, whose receptor is abundantly expressed on pericytes downregulates genes related to vascular permeability and promotes both endothelial–endothelial (VE-cadherin) and pericyte–endothelial cell (N-cadherin) interconnections

pericytes did not appropriately cover the brain capillaries in hyperlipidemic mice exposed to ischemia and, this was associated with attenuation of post-stroke angiogenesis [111].

Albeit indirectly, further supporting a role for pericytes in post-stroke angiogenesis, intravenous injection of a combination of smooth muscle progenitor cells and endothelial progenitor cells 1 day after MCA occlusion enhanced the angiogenesis and vessel maturation in the peri-infarct areas [122]. Since the adult bone marrow is considered to be a rich reservoir of pericyte progenitor cells, bone marrow-derived pericytes may be involved in post-ischemic angiogenesis [109, 123, 124]. Indeed, Kokovay et al. showed that, following brain ischemia, bone marrow-derived cells with a pericytic phenotype and expressing angiogenic factors were recruited to cerebral capillaries [109].

Angiogenesis is also essential to promote neurogenesis after stroke [122, 125, 126]. In fact, newly formed neurons have been found located near to the remodeled vessels [127], probably because vascular cells recruit and form a niche for neural stem cells [126, 128]. Since pericytes are essential in post-stroke angiogenesis and express factors that can induce neurogenesis as well as angiogenesis, pericytes may

also be involved in post-stroke neurogenesis [25, 129]. In vitro studies have clearly shown that the brain-derived pericytes have a potential to differentiate into neurons in response to trophic factors such as basic fibroblast growth factor, Sox2 and Mash1 [22, 130–132]. Pericytes obtained from ischemic MCA tissue of adult animals or pericytes cultured under ischemic conditions also showed capability to differentiate to cells of neural as well as vascular lineage [133].

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