



Small Vessels Are a Big Problem in Neurodegeneration and Neuroprotection

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Specialty section:

This article was submitted to
Neurodegeneration,
a section of the journal
Frontiers in Neurology

Received: 12 May 2019

Accepted: 01 August 2019

Published: 16 August 2019

Citation:

Erdener ŞE and Dalkara T (2019)
Small Vessels Are a Big Problem in
Neurodegeneration and
Neuroprotection.
Front. Neurol. 10:889.
doi: 10.3389/fneur.2019.00889

The cerebral microcirculation holds a critical position to match the high metabolic demand by neuronal activity. Functionally, microcirculation is virtually inseparable from other nervous system cells under both physiological and pathological conditions. For successful bench-to-bedside translation of neuroprotection research, the role of microcirculation in acute and chronic neurodegenerative disorders appears to be under-recognized, which may have contributed to clinical trial failures with some neuroprotectants. Increasing data over the last decade suggest that microcirculatory impairments such as endothelial or pericyte dysfunction, morphological irregularities in capillaries or frequent dynamic stalls in blood cell flux resulting in excessive heterogeneity in capillary transit may significantly compromise tissue oxygen availability. We now know that ischemia-induced persistent abnormalities in capillary flow negatively impact restoration of reperfusion after recanalization of occluded cerebral arteries. Similarly, microcirculatory impairments can accompany or even precede neural loss in animal models of several neurodegenerative disorders including Alzheimer's disease. Macrovasculature are relatively easy to evaluate with radiological or experimental imaging methods but they cannot faithfully reflect the downstream microcirculatory disturbances, which may be quite heterogeneous across the tissue at microscopic scale and/or happen fast and transiently. The complexity and size of the elements of microcirculation, therefore, require utilization of cutting-edge imaging techniques with high spatiotemporal resolution as well as multidisciplinary team effort to disclose microvascular-neurodegenerative connection and to test treatment approaches to advance the field. Developments in two photon microscopy, ultrafast ultrasound, and optical coherence tomography provide valuable experimental tools to reveal those microscopic events with high resolution. Here, we review the up-to-date advances in understanding of the primary microcirculatory abnormalities that can result in neurodegenerative processes and the combined neurovascular protection approaches that can prevent acute as well as chronic neurodegeneration.

Keywords: capillary, microcirculation, pericytes, oxygen extraction, flow heterogeneity, neuroprotection, neurodegeneration

INTRODUCTION

Cerebral microcirculation is a fundamental element for proper cerebral functioning since it is the main transport and distribution system for oxygen and nutrients that fuel the high and continuously changing metabolic demand of the brain tissue. The anatomical and physiological features of small vessels, which mainly consist of vasculature $<100\ \mu\text{m}$ in diameter (1), where most of the metabolic exchange takes place, are considerably different from large vessels and these differences make them difficult to study experimentally and virtually inaccessible in human beings. Over the last years, advances in imaging technologies and modeling made such investigations possible and increased the recognition of microcirculatory pathologies in acute and chronic neurodegenerative conditions, revealing novel mechanisms and potential therapeutic strategies. In this review, the structural and functional principles of cerebral microcirculation and its pathophysiological relevance will be discussed.

CAPILLARY DYSFUNCTION

The Complexity of Microcirculation

The brain is a vascular organ as much as it is neuronal. The anatomy of cerebral vasculature is unique and optimized to provide continuous blood supply to the brain. Extensive anastomoses between pial vessels on the surface ensure that extra blood can be shuffled to the activated brain area demanding more energy. As illustrated in **Figure 1**, progressive branching of penetrating arteries into a high-density meshwork of capillaries allows for adequate delivery of nutrients, such that there is a capillary within $10\text{--}20\ \mu\text{m}$ of every neuron (4, 5). The density of capillary mesh correlates with neuronal density and differs between cortical layers or brain regions. Unlike simplified illustrations, capillary networks have a complex structure, branching in various directions, forming loops, and curls to optimally match the tissue demand.

Tissue blood flow is provided by the pressure gradient across the microcirculatory bed vascular resistance, which is largely determined by the vascular diameter and blood viscosity (6). The focal brain activity is highly variable; hence, regional metabolic requirements change fast and continuously. This requires a precise spatial and temporal control of the local blood flow. Consequently, flow changes are tightly coupled to neuronal activity through a set of mechanisms integrated within the *neurovascular unit* (7, 8). This *neurovascular coupling* can be mediated by both feed-forward (neuronal activity itself directly regulates blood flow) or feed-back (the metabolic consequences of increased activity regulates blood flow) mechanisms (8, 9).

Studying microcirculation is challenging in animals as well as humans because of the structural and functional complexity of the system. This has caused a delay in understanding role of microcirculatory dysfunction in neurological disorders, which is more relevant than previously thought. The microcirculatory dynamics are fast and heterogeneous, so we need tools that can acquire data with high spatial (in microns) and temporal (in milliseconds) resolution. Most of the available information on

capillary flow and oxygenation is based on magnetic resonance imaging (MRI) as well as direct *in vivo* microscopic imaging that can be performed only in animals. Two-photon microscopy (TPM) through a cranial window in rodents provides high resolution angiogram of the capillary network and, red blood cell (RBC) flux and speed estimation within individual capillaries (12–14). Phosphorescence lifetime microscopy (PLIM) adds assessment of the oxygen with subcapillary resolution both in microvasculature and cerebral tissue (2, 15–22). Optical coherence tomography (OCT), which is sensitive to motion of scattering particles enable visualizing RBC flow label-free. Unlike TPM that images a limited area, OCT-angiography allows visualizing hundreds of capillaries simultaneously through the cortical mantle (10, 23–26). In MRI, a voxel of $1\ \text{mm}^3$ reflects merely an average of many capillaries but MRI has the advantage of imaging whole brain non-invasively (27).

Studies with these instruments have revealed that the increase in capillary blood flow occurs slightly before or in synchrony with upstream vessels, suggesting a direct role to the microcirculation to coordinate functional hyperemia response (29–32). Under resting state, most of the oxygen release into the tissue occurs at the precapillary arteriole level, while distal capillaries become an additional a site of oxygen extraction during functional hyperemia (2). Especially during baseline conditions, the capillary pO_2 distribution is highly heterogeneous, some capillaries have very low oxygen, while not necessarily making the tissue around them critically hypoxic but particularly vulnerable to fluctuations in blood flow and/or increase in metabolic demand (2, 16).

Microcirculation Plays an Active Role in Neurovascular Coupling

Models for blood flow simulation and oxygen transport / extraction (34–38) suggest that capillary flow patterns are important to determine tissue oxygen availability (37–39). While cerebral capillaries are normally almost always perfused with plasma during rest and activated conditions, the flow speeds of RBCs, like spatial oxygen distribution, are heterogeneous at baseline. This is caused by variability in capillary diameters, pressure gradients, and non-Newtonian fluid characteristics of blood, resulting in different RBC and plasma fractions in different capillaries that can affect the flow patterns (40) (**Figure 2C**). In a recent study, monitoring of capillaries frame-by-frame by OCT angiography revealed frequent temporary stalls that lasted for few seconds-minutes in individual capillaries (10) (**Figure 2A**). These temporal flow fluctuations can be caused by RBCs or white blood cells (WBCs) getting stuck or slowed down inside capillaries (10, 11, 41) because of their relatively large size compared to the capillary lumen (**Figure 2B**). They are more prevalent in capillaries closer to the distal end of the microcirculation, where pressure gradients are lower and RBCs are slower, and also where endothelial adhesion molecules like intercellular cell adhesion molecule-1 (ICAM-1) have higher expression (42). During functional activation, RBC flow speeds increase to make flow distribution in capillary bed more homogeneous and the dynamic stalls diminish (10) (**Figures 2D,E**). This homogeneous

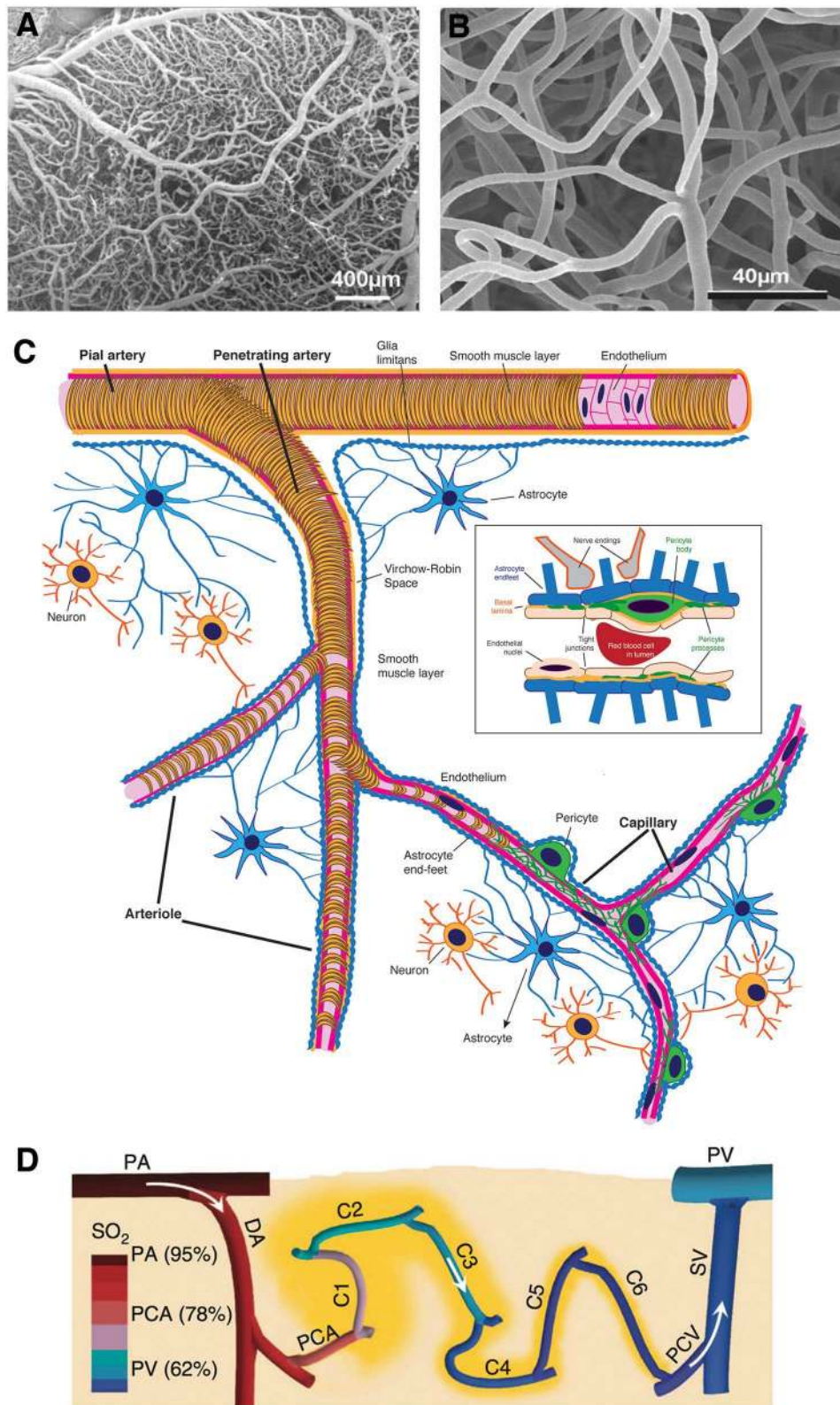


FIGURE 1 | Morphological features of cerebral microcirculation. **(A,B)** Dense external coverage of the surface by pial arteries and veins which are interconnected via anastomoses and collateral vessels then gives rise to a complex meshwork of capillaries, composed of segments about 60–70 µm in length. **(C)** Penetrating arteries *(Continued)*

FIGURE 1 | originating from pial vasculature dive into the cortical tissue without forming any further anastomoses. Immediately surrounded by the cerebrospinal fluid-filled spaces called *perivascular spaces* or *Virchow-Robin spaces* along their course, penetrating arteries then give rise an extensive tree of small vessels as they branch into arterioles (30–100 μm diameter), precapillary arterioles (10–30 μm), and capillaries (<10 μm), respectively. As arterioles turn into capillaries, the perivascular space disappears, making the capillary wall adjacent to the parenchyma. **(D)** Capillaries branch out for 5–6 times on average (2) as they release oxygen into the tissue, until they converge on postcapillary venules that drain into ascending veins. **(A,B)** reproduced by permission from: PNAS, Meyer et al. (3) ©2008 National Academy of Sciences. **(D)** reprinted by permission from: Nature Communications, Sakadžić et al. (2) ©2014 Springer Nature).

transformation in oxygenation and flow speeds is essential for optimum oxygen extraction. Because, in case of heterogeneous flows, some capillaries have higher flow velocity compared to the neighboring vessels and they act as thoroughfare channels, shunting highly-oxygenated RBCs through the microcirculation to leave little time for oxygen release (37). This is important because it has been shown that increasing overall arterial blood flow to a capillary bed with heterogeneous flow distribution can paradoxically worsen oxygen availability to the tissue (38, 43, 44). For this reason, functional blood flow responses in the brain need to be actively regulated at the capillary level and not solely by dilating arterioles. This function is realized by structural and metabolic collaboration between neurons, endothelia, astrocytes and pericytes, the building blocks of the neurovascular unit.

Endothelium is the common inner lining of all types of vasculature, including capillaries. In the central nervous system (CNS) tight junctions between endothelial cells form the critical layer of the blood brain barrier (BBB) that controls material passage into the parenchyma. In the healthy setting, endothelium prevents paracellular plasma leakage by tight junctions and there is a low level of transcytotic vesicle trafficking through endothelial cells (45, 46). Oxygen and carbon dioxide, on the other hand, can freely diffuse across the capillary wall bidirectionally (46–48), allowing rapid gas exchange. The endothelium, basal membrane, and astrocyte end feet together form the capillary wall and the BBB (49). Degradation of BBB either due to basal lamina changes, endothelial or astrocyte damage is a common feature of many neurodegenerative disorders which have microcirculatory pathologies. While there is no smooth muscle layer in the capillary wall, there is another contractile cell, the pericyte located at the periphery of microvessel wall and embedded into the basal membrane layers (50). Pericytes have a contractile apparatus, which is similar to that of smooth muscle cells. Pericyte processes concentrically surround the capillaries, enabling them to regulate the capillary diameter (51, 52). Pericytes have also been shown to contribute to BBB formation and maintenance, to immunological modulation, angiogenesis, and capillary reorganization (53–55). Pericytes therefore are attractive candidates for capillary-level control of neurovascular coupling. Indeed, pericyte relaxation during functional activation has been shown to precede or being closely coincident with functional hyperemia (30, 51). The strong interactions between pericytes and endothelial cells and astrocytes further underline their strategic role in microcirculation (56, 57).

Astrocyte end-feet around microvessels are critical elements for neuron-microvessel communication. Nearby neuronal activity is sensed by astrocyte processes as extracellular adenosine

triphosphate (ATP) and glutamate increase around synapses. Calcium signals triggered in astrocytes lead to cyclooxygenase activation and release of vasodilatory prostaglandins from end-feet surrounding the capillaries (58, 59). This is one of the basic mechanisms for translation of neuronal activity to vasoactive regulation. Other mechanisms involving metabolic and paracrine interactions within elements of the tightly integrated neurovascular unit are also present and contribution of each varies between brain regions. For example, astrocyte processes are also metabolically coupled to excitatory synapses; they rapidly breakdown glycogen to release lactate as an energy supply to neurons (60). An intriguing feature of the astrocyte network is the gap junctions between end-feet integrated to the capillary wall. Activity-induced calcium signaling can rapidly propagate around microvasculature to provide a coordinated response in a given area (59, 61–63). Capillary pericytes readily respond to the local release of vasodilatory prostaglandins (30). Endothelial cells, which are electrically coupled to other endothelial cells and to smooth muscle cells via gap junctions (64–67), have also been shown to propagate membrane depolarizations and calcium waves along the vasculature, releasing nitric oxide (NO) in consequence (68), adding to the coordinated microvascular regulation. Interneurons are also directly involved in vasoactive regulation. For example, stimulation of GABAergic interneurons has been shown to produce a biphasic blood flow response, hyperemia, followed by vasoconstriction, similar to the somatosensory activation response, by releasing NO and neuropeptide Y, respectively (69–72). Interestingly, red blood cells within the capillary lumen themselves can also take active role in capillary flow regulation by releasing ATP in response to focal acidosis, hypoxia, shear stress, or mechanical stimuli (73–76). The ATP release can be initiated by opening of pannexin channels or voltage-dependent anion channels in erythrocytes (75, 77). ATP, by binding to purinergic receptors can trigger NO or prostaglandin release from the endothelium (73) generating a local vasodilatory response to match the oxygen demand in the stagnated microenvironment (73).

The mechanisms of neurovascular regulation should be recognized in the setting of an integrated vascular network, where different levels of microcirculation may work in concert in addition to a tight compartmentalization. For example, arterioles appear to favor the vasoactive signals coming from interneurons coding the activity in a larger cohort of neurons in contrast to capillary regulation responding to very focal demand translated by adjacent astrocytes (69, 78). Arterioles also respond to signals coming up through the endothelia to accommodate the blood volume demand by the downstream microcirculatory bed (58, 59, 61–63).

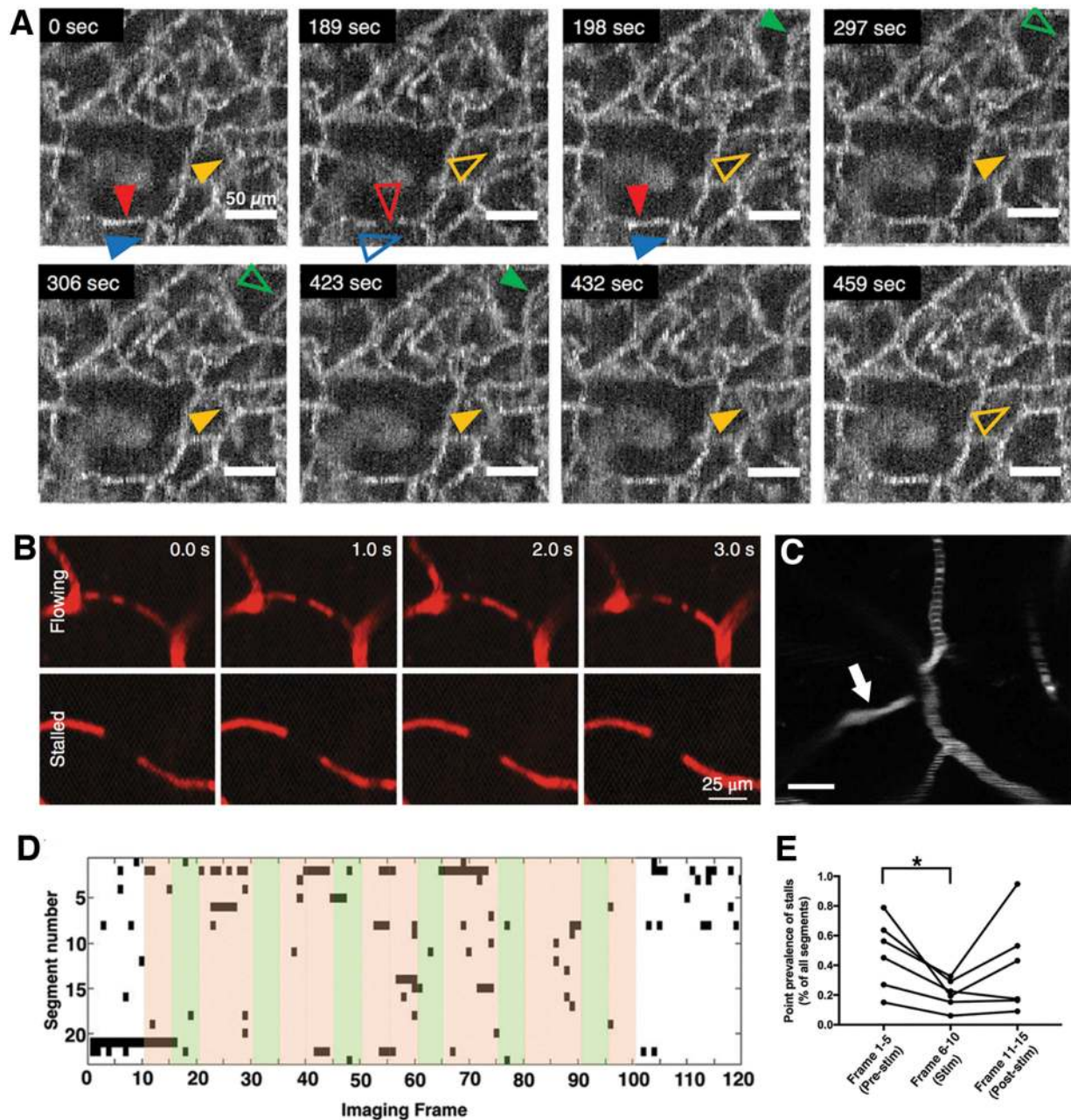


FIGURE 2 | Irregularity and heterogeneity in capillary flow **(A)** OCT angiogram time-series identify capillary segments with stalling red blood cells. Individual segments (arrowheads) with temporary interruptions of RBC flux simply lose OCT angiogram signal. Hollow arrowheads indicate a stalled capillary segment (10). **(B)** Two photon microscopy time series with fluorescent labeled plasma can identify flowing and stalled capillaries based on the motion of unlabeled cells as seen in black (11). **(C)** A single time-point two-photon angiogram of a set of capillary branches at $\sim 100 \mu\text{m}$ below cortical surface shows heterogeneous distribution of RBC flux. Segments with higher flow have thinner and denser RBC-bands whereas slower flow is indicated by thicker and more scarce bands. One segment (arrow) has no RBCs flowing but is still filled with fluorescent labeled plasma. Scalebar: $20 \mu\text{m}$ **(D)** Matrix plot of individual stall events in a region of interest, acquired during a functional stimulation experiment, with a frame period of 9 s. Every black point denotes a stall in a particular capillary. Green shades indicate whisker stimulation. **(E)** The frequency of capillary stalls is dynamically modulated during functional stimulation; stall prevalence was significantly lower during functional hyperemia. *Statistical significance ($p < 0.05$). **(A,D,E)** reproduced by permission from: JCBFM, Erdener et al. (10); **(B)** reprinted by permission from: Nature Neuroscience, Cruz Hernandez et al. (11), ©2019 Springer Nature).

Microcirculatory Failure

Since oxygen availability in the tissue relies on the distribution of capillary flow patterns and their heterogeneity, the absolute

quantitative level of cerebral blood flow (CBF) may not always reflect accurate supply-demand match at microscopic level. In routine clinical practice of cerebrovascular medicine, main focus

is on the evaluation of adequate arterial flow and luminal patency, as they can be easily imaged by widely available radiological techniques. In the setting of a CBF drop, particularly to ischemic levels (below ~ 20 ml/100 g/min in humans) (79), there is a prominent decrease in tissue oxygen tension paralleled by a relative increase in oxygen extraction. However, experimental and computer simulation data indicate that the tissue oxygen availability may be compromised even in the absence of a flow-limiting condition (37, 38). This arises from “capillary dysfunction,” causing excessive flow heterogeneity in the capillary bed and failing to homogenize in response to increasing functional demand. This heterogeneity causes certain capillary segments to transit oxygenated RBCs too fast for enough oxygen to be released to tissue (36, 44). A classic example of this phenomenon is the hyperperfusion syndrome (38, 80, 81), a temporary excessive hyperemia after recanalization of an occluded cerebral artery, which paradoxically results in low tissue oxygenation (38). Very slow flux rates in some capillaries may also lead to a reduced oxygen delivery due to insufficient number of RBCs transiting. Any factor introducing irregularity to the capillary flow, results in a higher standard deviation in distribution of RBC velocities and/or transit times across the microvascular bed, i.e., the capillary transit time heterogeneity (CTH). However, it should be noted that increased CTH may passively increase with high mean transit time (MTT), the average time that contrast bolus traverse between arterial and venous ends (43). Importantly, the relative transit time heterogeneity (CTH/MTT), i.e., the coefficient of variation of transit times, can distinguish between true microcirculatory heterogeneity and a passive increase in CTH with low CBF and elevated MTT. The relationship between CBF, CTH, oxygen extraction fraction (OEF), and tissue oxygen can be evaluated with computational models (36, 82, 83). These models show that, CTH is, counter intuitively; homogenized with very low flow rates to maximize O_2 extraction in poorly perfused tissue (37, 82). The detailed evaluation of mathematical models is beyond the scope of this review and the reader is referred to excellent papers on this subject (84, 85). MRI data can be used to prepare MTT, CTH, and OEF maps with use of appropriate mathematical models (27). These tools have been useful for evaluation of capillary dysfunction in acute ischemic stroke and Alzheimer’s disease patients (36, 38, 43, 82, 86).

The capillary dysfunction can arise from almost any element within the neurovascular unit. A thin ($0.5 \mu\text{m}$) glycoprotein coating in the luminal side of the endothelium, called glycocalyx, facilitates passage of blood cells (87–89). Glycocalyx can degrade very fast with excessive inflammatory stimuli like lipopolysaccharides, hyperglycemia, ischemia, and oxidative stress (90). A degraded glycocalyx exposes underlying endothelium to physical interaction with blood cells. This usually results in a higher degree of cellular adhesion. Each cellular plug, whether permanent or transient, can then increase resistance in that particular segment, change pressure gradient, flux, and hematocrit distribution in nearby capillary bed and cause irregular hemodynamics. A relative increase in hematocrit in other capillaries within the same network may increase the tendency for plugging.

Endothelial cell dysfunction can significantly contribute to dysregulated capillary flow. This dysfunction includes a reduction in endothelium-derived nitric oxide availability. Moreover, gap-junction uncoupling between endothelial cells increases functional shunting by introducing irregularities to the microvascular cross-sectional profiles, caused by desynchronized regulation of smooth muscle or pericyte tone (91, 92). A dysfunctional endothelium is also more prone to leukocyte adhesion, as nitric oxide is a well-recognized modulator of leukocyte adhesion (92–94). Leukocyte plugs and resultant flow stalls in capillaries can unfavorably affect flow patterns and, the release of leukocyte-derived oxygen radicals or cytokines can damage the endothelium and overlying glycocalyx. BBB breakdown may accompany a damaged and dysfunctional endothelium. Endothelium, besides these barrier-forming and material exchange functions, is also involved in immunological regulation, leukocyte adhesion and regulation of thrombosis as well as vascular tone (95).

Pericytes maintain the BBB, interact with immune cells (96) and, since they are contractile, regulate capillary flow distribution within the microvascular network. Both constriction or dilation of capillaries mediated by pericytes can result in stalls in cellular flow or functional shunts (32). Pericytes contract or constrict with changes in oxygen tension, with exposure to reactive oxygen radicals, noradrenaline, ATP and endothelin (28, 30, 53, 97), while glutamate, adenosine, lactate, nitric oxide, and C-type natriuretic peptide cause relaxation (30, 97–99). Besides active contractile changes, loss of pericytes themselves can cause capillary dysfunction (100). Amyloid-beta has been shown to cause pericyte degeneration (101, 102), which may be a possible link between neurodegenerative disorders and microcirculatory dysfunction. Hyperglycemia directly causes pericyte death or migration, hence, leaky newly-formed microvessels are typical features of diabetic retinopathy (99, 103). Pericytes have been shown to migrate along or away from vasculature following traumatic injury (104, 105). With pathological stimuli, pericyte process coverage over capillary wall can decrease, further disrupting microvascular physiology (106, 107).

Finally, the physicochemical properties of plasma or blood cells themselves can introduce microcirculatory heterogeneity. As noted above, RBCs and WBCs have large diameters compared to capillaries therefore they need to squeeze through the narrow capillary lumen, significantly making passage challenging and vulnerable to disturbances (108). Even subtle capillary constrictions can therefore dramatically affect RBC perfusion distribution in a capillary network (36, 82). Changes in cell count, size, stiffness, flexibility, and adhesion can affect the capillary transit (8). In experimental models of polycythemia vera and essential thrombocythemia, it has been shown that individual capillaries are clogged by RBCs, and then platelets, affecting the CBF (41). Rheological properties, like deformability of RBCs can affect microcirculatory efficiency as seen in diabetes (109). Blockade of capillaries by activated neutrophils are also observed in cerebral ischemia (110–113). Dehydration and elevated homocysteine can increase plasma viscosity and/or hematocrit, causing formation of RBC aggregates, and capillary plugs (38, 114). It should be noted that plasma may continue to

flow though even when there is no cellular flow in a capillary, unfavorably affecting tissue survival as plasma provides glucose to the hypo-oxygenized tissue, promoting lactate production and tissue acidosis (28, 115–117).

The tissue can tolerate a certain degree of capillary dysfunction by regulating upstream arteriolar flow (either increasing or decreasing) to optimize oxygen extraction (37, 44, 118–120). A reduction in resting CBF and suppression of functional hemodynamic responses can be a part of this compensation (37, 121). But after a certain degree of CTH, usually accompanied by a higher level of capillary diameter irregularities, glycocalyx damage, elevated blood viscosity, leukocyte adhesion, and excessive red blood cell stalls, the supplied oxygen cannot sustain neuronal homeostasis any more (36). At this level of severe capillary dysfunction, where CBF is close to the ischemic threshold, slight changes in metabolic needs, systemic blood pressure, leukocyte count, or blood viscosity may cause appearance of hypoxic/ischemic neurological symptoms (36, 37). These events can also cause irreversible morphological changes in microcirculation, like capillary pruning, or appearance of string capillaries with no functional lumen (122–124). It should be noted that all these pathological events described above could occur in the absence of a flow-limiting condition, like arterial occlusion, or stenosis.

MICROCIRCULATORY DYSFUNCTION IN NEURODEGENERATION

Small Vessel Disease

Small vessel disease is a clinical and imaging phenomenon caused by different etiologies and characterized by pathological changes in vasculature with a diameter <100 μm , including, arterioles, venules, and capillaries (1, 125). It is a common cause of cognitive impairment, gait problems and disability in the elderly (125). Uncontrolled hypertension or diabetes are among the leading risk factors for this chronic vasculopathy characterized with concentric smooth muscle thickening, especially in penetrating vessels as well as pericyte degeneration, basal membrane thickening, endothelial, and astrocyte end-feet swelling in capillaries (38, 126–129). Pro-inflammatory conditions in the endothelium increases tendency to leukocyte adhesion and activation in this pathologic setting (130–134). The slowly progressive worsening of microcirculatory structure and function may result in neuronal loss, brain atrophy, and the white matter changes detected as leukoaraiosis or diffuse white matter hyperintensities with MRI (135, 136). While capillary-level damage may insidiously progress over time, acute vascular events, like spontaneous rupture, or thrombosis of already damaged arteriolar branches (137–141), leading to stepwise clinical deterioration (1). It is not surprising that either the chronic progressive vasculopathy or acute ischemic events superimposed on this is a primary cause of microcirculatory dysfunction, the details of which are outlined in the next section.

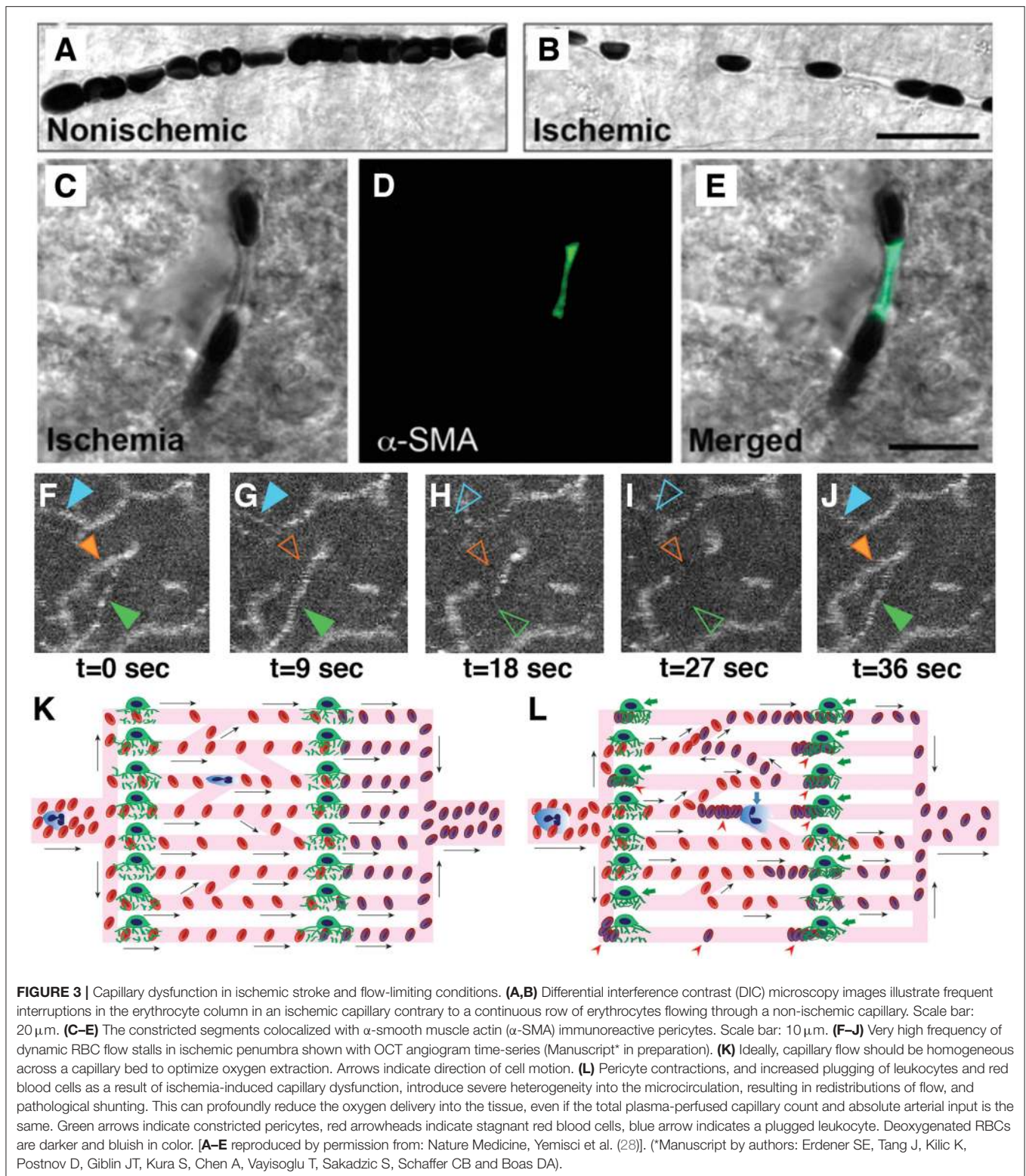
Ischemic Stroke

Currently, the only approved treatment of an acute ischemic stroke is prompt recanalization within few hours either by

mechanical thrombectomy or thrombolysis with intravenous or intra-arterial tissue plasminogen activator. At the present, this treatment approach only takes large vessel recanalization into account. However, tissue reperfusion is usually incomplete, hence oxygen extraction is not optimal even after complete recanalization (38). In the ischemic brain, pericytes contract in response to hypoxia and reactive oxygen species (28). This luminal narrowing and irregularities can easily block cellular passage, while plasma may continue to flow. Recanalization of the artery does not resolve this microvascular dysfunction as the capillary constrictions are sustained, leading to the no-reflow phenomenon (142, 143) (**Figures 3A–E**). The quality of capillary perfusion after recanalization is indeed a better indicator of functional outcome than recanalization itself (144, 145). If the capillary flow patterns are extremely dysfunctional and heterogeneous, the recanalization may in fact introduce more functional shunting, paradoxically reducing the oxygen availability (36, 38, 146).

In the ischemic brain, activated neutrophils can also temporarily plug capillaries with their large size and stiff cytoplasm, and can disturb the RBC transit (108). As a clinical evidence, leukocytosis and a high neutrophil fraction are bad prognostic factors in ischemic stroke (147–150). Stroke incidence as well as stroke-related mortality is higher during acute infections (36, 151). The dynamic microcirculatory dysfunction may continue after stroke onset, potentially contributing to infarct expansion over the subsequent days despite recanalization (152–155). One essential way to prevent infarct growth appears to reverse any capillary flow disturbance in addition to large artery recanalization. It is also becoming clear that the definition of ischemic tissue based solely on the level of CBF decrease is insufficient and the capillary flow patterns must also be taken into account (36).

The ischemia-induced microvascular dysfunction progresses in a graded fashion. With a temporary decrease in perfusion pressure in a normal and compliant microvasculature, the cells transiting the microvasculature slow down, since there is less driving force due to lower pressure gradient. Accordingly, MTT values increase. The CTH calculated based on the standard deviation of transit times in multiple pathways across the capillary bed proportionally increases as well. Thereby, the relative transit heterogeneity ($\text{RTH} = \text{CTH}/\text{MTT}$) remains unchanged and oxygen delivery to the tissue can still be sufficient to match the demand. However, with ongoing ischemia, structural irregularities, like constrictions, and glycocalyx damage in capillaries emerge, making cellular passage complicated (**Figures 3F–L**). This time, the increase in CTH will be higher than what can be mathematically expected from prolongation of MTTs. The disproportional increase in CTH will yield to higher RTH values, pointing to a specific dysfunction in microcirculation that can result in suboptimal oxygen extraction for the ischemic tissue. The above simplified progressive microcirculatory deterioration is the most likely scenario taking place in the ischemic penumbra, corresponding to a potentially salvageable tissue with timely and efficient recanalization, whereas it can also undergo permanent damage with increasing microvascular damage and oxygenation failure



(82). In the most severe form of ischemia, complete capillary occlusions that do not allow even passage of plasma emerge. This end-stage level of capillary dysfunction, paradoxically leads to homogenization of capillary transit times measured from

remaining patent ones in the severely ischemic tissue that is destined to infarct regardless of recanalization (82). This low CTH probably reflects flow in maximally dilated flowing capillaries and does not take into account the occluded ones.

The phrasing may at first sound at odds with the capillary flow homogenization during normal functional hyperemia that provides optimal oxygen extraction (37). Although it may be a similar neurovascular coupling effort for the tissue to optimize blood flow using the capillaries left available (82), the absolute amount of blood passing through the tissue this time is simply very low, decreasing cerebral blood volume below the viability threshold (156).

Chronic cerebral hypoperfusion, even if it doesn't cause full-blown ischemia, can have long-term effects on microcirculatory structure. Experimental OCT data in mice with carotid artery occlusion show a local micro-heterogeneity of capillary flow and oxygen supply even at non-ischemic global blood flow levels (49). The functional changes are accompanied by histological signs of microglia and astrocyte activation along with capillary dilatation, remodeling, increased tortuosity, and even amyloid-beta accumulation (157–159). These structural pathologies can further contribute to the progression of capillary dysfunction. Recently, severity of carotid stenosis have been associated with accumulation of cortical cerebral microinfarcts and poor cognitive performance, even in patients who did not experience acute ischemic stroke (160). Preoperative capillary transit time heterogeneity measured by MRI, an indicator for capillary dysfunction, predicts the functional independency (i.e., Modified Rankin Scale) following endovascular treatment in patients with symptomatic bilateral high-grade carotid stenosis (161).

Subarachnoid Hemorrhage

Subarachnoid hemorrhage shares many common pathophysiological features with cerebral ischemia, because the blood products in cerebrospinal fluid (CSF) can trigger arteriolar and microvascular vasospasms (162). Hemoglobin breakdown products and concomitant formation of reactive oxygen species by iron released from hemoglobin (163) as well as release of vasoconstrictive mediators like endothelin-1 and thromboxane into the CSF may play roles in vasospasm (39, 164). Vasospasms emerge usually a few days after the initial hemorrhage. Like in cerebral ischemia, there is profound increase in capillary transit time heterogeneity after subarachnoid hemorrhage, caused by microarteriolar constrictions (165), reactive oxygen radicals, astrocyte end-feet swelling, leukocyte increase, and activation (39). There are numerous capillaries either without RBCs but with plasma, or with stationary RBCs in them (166). Capillaries with smaller diameters have been shown to have less probability to be perfused with RBCs (167). Because of these disturbances in microcirculation, attempts to relax the vasospasm in large arteries are not very effective. The microcirculatory pathology may begin even before gross vasospasm settles in. Within a few minutes after experimental subarachnoid hemorrhage, there is a heterogeneous slowing down of capillary flow with prominent irregularities, even though there is some dilation in penetrating vessels and precapillary arterioles (168, 169). Dilatory response to CO₂ is lost and an inverse neurovascular coupling response, i.e., constriction instead of expected hyperemia with sensory stimulation is observed 24 h after experimental subarachnoid hemorrhage (170). Whether this flow suppression is an outcome of pathologically high capillary heterogeneity or is a

compensatory CBF decrease to optimize oxygen extraction is currently unknown (39).

Traumatic Brain Injury/Chronic Traumatic Encephalopathy

Ischemic injury is a well-known histological hallmark of traumatic contusion injuries (171). A reduction in global CBF almost always accompanies a contusion or traumatic hematoma. If the injury is severe, vasospasms in large vessels can emerge similar to subarachnoid hemorrhage, causing multifocal infarcts. Elevated intracranial pressure due to traumatic injury in animal models caused severely disturbed capillary flow and limited oxygen and solute extraction due to heterogeneity and shunts in the capillary bed (37, 172). This can be partly explained by a direct negative effect of increased intracranial pressure on cerebral perfusion pressure. The decrease in CBF can last for 12 h and functional blood flow responses are usually suppressed during this period (173, 174). Histologically, there is evidence of astrocyte end-feet swelling, pericyte contraction, and increased expression of smooth muscle actin (174). Also, pericytes have been shown to detach from the vessel wall early after the traumatic insult (105), which might contribute to blood-brain dysfunction in capillary bed (175). Capillaries have been shown to be the major site of vascular leakage in animal models after cortical trauma (176). Increased inflammatory protein expression and activation of leukocytes promote cellular adhesion, therefore limiting RBC passage and causing functional capillary stalls and shunts (108, 174). Leukocytosis, like in ischemic stroke, is associated with poor outcome following traumatic brain injury (172).

Interestingly, a gross contusion, hematoma, or intracranial pressure increase is not necessary for microcirculatory abnormalities to develop. A single mild concussion in mice can also decrease CBF 30–40% up to 24 h, as measured with diffuse correlation spectroscopy (177). With repetitive injuries, the oligemic response can extend to 72 h (177). The microcirculatory disturbances induced by mild injuries are independent of increases in intracranial pressure (178). Instead, cortical spreading depolarizations (CSD) multifocally triggered after such insults correlate with neuronal injury and cerebral microbleeds (172). CSDs are self-propagating waves of intense neuroglial depolarization, followed by prolonged suppression of neural activity. Whereas, CSDs associated with migraine aura are benign, repetitive CSDs can cause tissue injury in the setting of subarachnoid hemorrhage or cause infarct expansion during cerebral ischemia, in the form of peri-infarct spreading depolarizations (179–184). In a healthy brain, CSDs can initiate a hyperemic response followed by a long-term oligemia but, in an already ischemic brain, the hyperemia is reversed to oligemia during the depolarization phase (185). CSD waves cause sustained disturbances in capillary flow, causing massive, and heterogeneous changes in erythrocyte velocities (186). Pericytes contract during CSD in response to an increase in cytoplasmic calcium (187). Neurovascular coupling can disappear for hours, causing a supply-demand mismatch, contributing to tissue injury (186, 187). The reduced (inverse)

CBF response to increased metabolic load during massive depolarization could help increase O₂ extraction by slowing flux rates (186).

Repetitive concussions are associated with chronic traumatic encephalopathy (CTE), a cause of early onset dementia and psychiatric disorders especially in boxers or American football players and also in military veterans exposed to blast injuries (188). Autopsy brain samples from CTE patients and also rodent models have evidence of microcirculatory pathology, characterized with swollen astrocytic end-feet and processes forming tangles around capillaries, blood-brain barrier breakdown, and perivascular deposition of hyperphosphorylated tau especially in deep cortical regions (189–192). Even a single blast exposure or concussive cortical impact in mice has been shown to trigger a progressive tauopathy with evidence of microvascular injury and neurodegeneration, which started in the area of exposure first but spread distally over months (193–195).

Alzheimer's Disease

A reduction in CBF is observed early in Alzheimer's disease (AD) patients and this has been proposed as a predictor of progression to overt AD from mild cognitive impairment (196–198). Many vascular risk factors like coronary heart disease, dyslipidemia, hypertension are associated with AD (199). The underlying mechanisms and possible impact of the reduction in CBF and microcirculatory abnormalities in the pathogenesis of neurodegeneration are under investigation to better characterize the cause-effect relationship. In mouse models, impaired cerebral perfusion has been shown to stimulate amyloid-beta deposition. Tau protein can also be hyperphosphorylated under hypoxic conditions, even after brief and temporary episodes (200). After global brain ischemia, expression of tau protein increases in CA1 area of hippocampus in mice, one of the most severely affected areas in AD related to memory functions (201). Interestingly, perivascular zones around penetrating arterioles, the bottlenecks of the cerebral blood supply, are one of the initial areas showing tau and amyloid beta deposition (202).

Electron microscopy has identified capillary wall damage, basal membrane thickening and pericyte degeneration in autopsy brains from AD patients (203). In MRI studies, cognitive performance and level of cortical atrophy were found to be associated with low CBF, high CTH, and low oxygen extraction (86, 204). Similar dynamic abnormalities have also been found in transgenic AD mice, with insufficient flow homogenization, lower resting CBF, and reduced cortical oxygen availability (205).

A recent study using *in vivo* two photon microscopy in transgenic mice with excessive amyloid deposition demonstrated that an increased number of cortical capillaries had stalled blood flow due to neutrophils adhered to vessel wall (11) (Figures 4A–C). These stalls have also been found to correlate with amyloid deposition and, targeting neutrophil adhesion improved blood flow and cognitive performance (11). Another longitudinal study tracked obstructed capillaries and identified a VEGF-dependent pruning mechanism with regression of endothelial cells, causing uncompensated capillary loss (206). Perivascular macrophages, in response

to amyloid-beta, can release reactive oxygen species that can aggravate microcirculatory dysfunction (207). Mouse models of increased hyperphosphorylated tau expression revealed capillary constrictions surrounded by swollen astrocyte processes (208), formation of abnormal spiraling capillary morphologies with impaired blood cell flux (33) (Figures 4D,E).

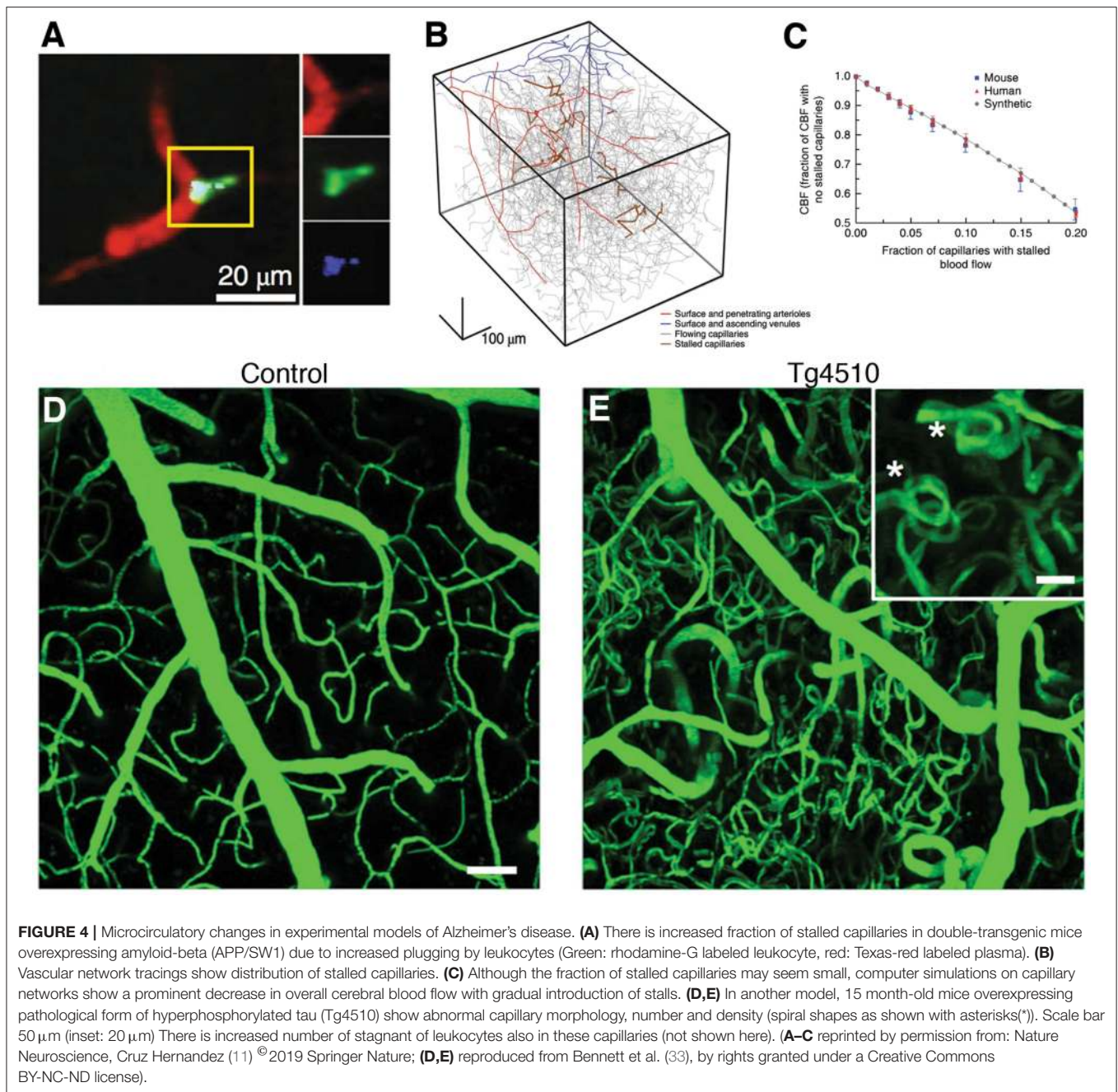
Transgenic mice with pericyte deficiency have been shown to have impaired capillary perfusion and neurovascular coupling, reduced oxygen supply, blood-brain barrier disruption, white matter degeneration and, importantly, progressive AD-like pathology (including progressive tauopathy and neuronal loss), resulting in accelerated cognitive decline (31, 100, 209–211). A recent study in AD patients showed reduced levels of platelet-derived growth factor-B, a pericyte marker, in precuneus, a cortical area affected in AD (101). We do not know exactly how pericytes degenerate progressively in those conditions, but there are some clues. For example, external introduction or overexpression of amyloid-β can damage pericytes directly (209, 212, 213) and conversely, pericytes can themselves be a zone of production of amyloid-β-associated proteins (214). Hypertension, dyslipidemia, and ApoE4 isoforms, all independent risk factors for AD, can also impair pericyte structure and function, that can potentially initiate a neurodegenerative phenotype (215–220). Pathogenic hyperphosphorylated tau, on the other hand, promotes BBB injury directly (221); the possibility that this injury may be related to pericyte damage, however, remains to be investigated.

Other Chronic Neurodegenerative Diseases

Accumulating evidence suggests that several other progressive neurodegenerative conditions may also involve primary microcirculatory pathologies, Like AD and CTE as discussed above, other tauopathies such as corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and amyotrophic lateral sclerosis (ALS) exhibit possible microvascular involvement (222).

In Parkinson's disease, both experimental and clinical data suggest microvascular involvement. Capillary loss and abnormal fragmented capillaries or endothelial clusters can be detected in substantia nigra, cerebral cortex and brain stem in pathological brain samples (223). There are also compensatory new vessel formations, which are possibly immature and leaky. In animal models produced by MPTP treatment, decreased capillary density or formation of abnormal endothelial clusters have been reported as observed in autopsy brains (224).

CBD and PSP are two atypical and rapidly progressive forms of parkinsonism. In postmortem brains of CBD and PSP patients, prominent accumulation of hyperphosphorylated tau in close proximity to vasculature, astrocytic proliferation, and hypertrophy as well as reduced capillary diameter have been detected (225, 226). These findings are difficult to test experimentally since there are no established animal models of these conditions. There are transgenic mouse models, however, available for another tauopathy, ALS, with mutations in superoxide-dismutase (SOD1) (227), tar DNA binding protein



(TARDBP/TDP43) (228), fused in sarcoma (FUS) (229), or C9orf72 (230) genes. SOD1 mouse model exhibits progressive decrease in the anterior gray matter of the spinal cord, beginning from the presymptomatic stage before neuronal degeneration, and is accompanied by a reduction in capillary diameter and density as well as slowing down of RBC flow (231). The flow-metabolism coupling is also disrupted as glucose utilization in contrast to the oxygen availability is increased especially in early stages. These findings become more profound as the disease progresses (232). At present, there is no reported data on microvascular features of mice with other ALS-related mutations, however, future studies might provide deeper insight.

In ALS patients, the cerebral blood flow is decreased especially in the prefrontal cortex (233, 234) and a clinical picture of frontotemporal dementia can accompany ALS (235). In ALS patients, the blood-spinal cord barrier is also damaged and extravasated hemoglobin and hemosiderin deposits can be detected in perivascular areas (236). In ALS transgenic mice, there is data showing capillary pericyte degeneration and blood-spinal cord barrier leakage, which can precede motor neuron degeneration (231, 237).

Finally in epilepsy, a group of disorders characterized with chronic repetitive seizures, progressive neurodegeneration can occur over time and also a single prolonged seizure can

initiate such degeneration (238, 239). This has previously been attributed to glutamate-induced excitotoxicity (240). Since there is usually increased blood flow in seizure foci (241–243), one would not readily suspect from impaired oxygenation as a possible cause of neuronal loss. But as discussed above, capillary dysfunction may occur in the setting of increased cerebral blood flow (38). An elegant study with *in vivo* two-photon microscopy, however, identified individual capillaries with pericyte contractions and accompanying stalls in blood flow in hippocampus of epileptic mice during seizures and those abnormal capillary vasodynamics created an environment with irregular capillary flow and possibly microscopic hypoxia, which was associated with neurodegeneration in close proximity of constricted capillaries (244).

POSSIBLE THERAPEUTIC APPROACHES TO IMPROVE CAPILLARY FLOW AND OXYGENATION

The above-discussed findings strongly suggest that, to slow down or to reverse the acute and chronic neurodegeneration, the microcirculatory structures and dynamics must also be taken into account. Interventions to improve the microcirculation need to have specific targets to homogenize capillary flow and optimize oxygen and glucose extraction that would meet the metabolic needs at all times. These approaches should complement interventions for the macrovessels. Any attempt to increase the cerebral blood flow without correcting microcirculatory disturbances may aggravate heterogeneous shunting and paradoxically worsen oxygen extraction (38).

Reactive Oxygen Species and Pericyte Contraction

Various scavengers can target reactive oxygen species that are responsible for vasospasms as well as capillary pericyte contraction and endothelial dysfunction. One of these, N-tert-Butyl- α -phenylnitron (PBN), has been shown to improve microcirculation in a mouse model of cerebral ischemia, improving tissue injury and functional outcome (245). Since NO depletion may be contributory to capillary constriction, nitric oxide inhalation as well as nitrite infusion have been suggested to improve microcirculation and tissue oxygenation in various models of neurovascular disorders (246–252). Like nitric oxide, adenosine is involved in active physiological regulation of microvascular diameter and perfusion (58, 253). Adenosine is endogenously released as a metabolic end product of cerebral activity and external adenosine administration increases capillary diameter by relaxing pericytes in both myocardial and cerebral microcirculation, under ischemic conditions (99, 254–256). A major problem with nitric oxide donors and adenosine-based therapies is their systemic side effects, including systemic cardiovascular and respiratory changes (257–260). Novel strategies that allow targeted cerebral delivery of such potent drugs, like squalenoyl adenosine nanoassemblies (99), or nanoparticulated nitric oxide donors (261), and also inhaled nitric oxide that does not have systemic cardiovascular side

effects, can be highly useful to promote clinical translation of this pharmacologic potential.

Capillary pericytes constrict in response to adrenergic receptor activation and with endothelin, therefore their antagonists could be considered as potential therapeutic targets (51, 262). Pericyte constriction is calcium-dependent as it involves actin machinery (30). Voltage-gated calcium channel blockers have been shown to decrease pericyte constrictions and also pericyte death, improving capillary transit time homogenization (262, 263). Ca^{+2} -activated chloride channels can also potentiate pericyte depolarization and constriction and can be another target (262, 264). Rho-kinase pathway inhibition can reverse abnormal pericyte contractions (265, 266) and this approach has been found to be neuroprotective in mouse models of cerebral ischemia (267–269).

Glycocalyx Integrity

Shedding of glycocalyx can impair homogeneities in RBC flux and increase endothelial-leukocyte adhesion. Glycocalyx has been protected by anti-thrombin administration in a rat sepsis model and this treatment decreased leukocyte adhesion, improving microcirculatory blood flow in mesentery (270, 271). Avoiding hyperglycemia is also beneficial for glycocalyx integrity (271). Similarly, a hydroxyethyl starch improved lung microcirculation by preventing experimental glycocalyx degradation by heparinase (90). Therefore, restoring glycocalyx integrity can be another potential treatment approach.

Blood Viscosity and Hematology

Since increased leukocyte count, activation, and increase in hematocrit (either due to higher RBC number or dehydration) increase capillary clogging, stalls and heterogeneity, blood viscosity, and endothelial adhesion could be one of the possible targets. Phosphodiesterase inhibitors like pentoxifylline increase RBC flexibility and decrease blood viscosity (272, 273) and they can indeed improve microcirculatory profile while their direct effects on augmenting NO activity might be another contributor to this (274). Prevention of dehydration and concomitant infections could also be beneficial for a regular and optimal capillary flow (38). These two factors are among the most common triggers of delirium and cognitive fluctuations in elderly patients with dementia (275).

Decreasing leukocyte count has been reported to provide a beneficial outcome in animal models of cerebral ischemia (276). A monoclonal antibody against Ly6G surface proteins on neutrophils has improved microcirculation by reversing neutrophil-mediated capillary stalls in mice, increased blood flow and behavioral scores acutely in mice, an effect attributed to modulation of neutrophil adhesion in capillaries (11). The same antibody has been found to be beneficial in mice with subarachnoid hemorrhage (277) and after stroke in hyperlipidemic mice (278). The exact physiological role and endogenous receptor of Ly6G is currently unknown (279) but these experimental data require further investigations.

Other innovative approaches to modulate rheological parameters of blood cells are continuously being developed.

Drag-reducing polymers improved flexibility of RBCs, decreasing cell stasis in capillaries, and plasma separation in capillary branching points, improving capillary flow heterogeneity in cerebral and myocardial ischemia models (280–282). Uptake of plasma-derived extracellular vesicles by RBCs have recently been shown to increase their deformability (283, 284).

CONCLUSION

Our review can help the recognition that many microvascular pathogenetic mechanisms are shared across a variety of acute and chronic neurological conditions and these mechanisms are not simply by-standers but are actually role-players, emerging in very early phases of neurodegenerative conditions. With advancement of optical microscopy, MRI-based imaging tools having higher spatiotemporal resolution as well as computer models, the physiology and vulnerabilities of the microcirculation will be better elucidated. Investigators in the field should not be surprised to see the introduction of previously unknown details, which will eventually lead to much better

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neuroprotectants for many neurological disorders within the next decades.

AUTHOR CONTRIBUTIONS

ŞE drafted the manuscript and prepared the figures. TD drafted and critically revised the manuscript.

FUNDING

TD work is supported by Turkish Academy of Sciences. ŞE work is supported by Turkish Neurological Society and The Science Academy, Turkey.

ACKNOWLEDGMENTS

The authors would like to thank Dr. David A. Boas (Boston University) and Dr. Sava Sakadzic (Massachusetts General Hospital) for their supervision of ŞE postdoctoral studies and helpful discussions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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