Pericyte mechanics and mechanobiology

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REVIEW

SUBJECT COLLECTION: MECHANOBIOLOGY

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ABSTRACT

Pericytes are mural cells of the microvasculature, recognized by their thin processes and protruding cell body. Pericytes wrap around endothelial cells and play a central role in regulating various endothelial functions, including angiogenesis and inflammation. They also serve as a vascular support and regulate blood flow by contraction. Prior reviews have examined pericyte biological functions and biochemical signaling pathways. In this Review, we focus on the role of mechanics and mechanobiology in regulating pericyte function. After an overview of the morphology and structure of pericytes, we describe their interactions with both the basement membrane and endothelial cells. We then turn our attention to biophysical considerations, and describe contractile forces generated by pericytes, mechanical forces exerted on pericytes, and pericyte responses to these forces. Finally, we discuss 2D and 3D engineered *in vitro* models for studying pericyte mechano-responsiveness and underscore the need for more evolved models that provide improved understanding of pericyte function and dysfunction.

KEY WORDS: Pericyte, Cytoskeleton, Contractility, Cell migration, Cell mechanics, *In vitro* models

Introduction

Pericytes are mural cells of the microvasculature that are embedded in the vessel basement membrane (BM), where they surround endothelial cells (ECs). Pericytes are essential for both the formation and maintenance of the vasculature (Bergers and Song, 2005). There is also mounting evidence that pericyte dysfunction plays a critical role in many microvascular diseases (Cathery et al., 2018), including tumor angiogenesis (Bergers and Song, 2005; Hodges et al., 2018), diabetic pathologies (Ferland-McCollough et al., 2017) and neurological disorders (Cheng et al., 2018; Hirunpattarasilp et al., 2019), most notably Alzheimer's disease (Sagare et al., 2013; Schultz et al., 2018; Sweeney et al., 2016). Pericytes were first discovered by Eberth and Rouget a few years apart at the end of the 19th century. The term 'pericyte' – 'peri' meaning around and 'cyte' for cell – was coined by Zimmerman in 1923 when he published a detailed morphological study of these cells across multiple mammalian species (Zimmermann, 1923). Interestingly, definitive identification and categorization of pericytes remains a major challenge due to the lack of specific biological markers (Smyth et al., 2018). Consequently, the consensual definition of a pericyte today is a cell that surrounds microvascular ECs, is embedded in the vascular BM and has a protruding cell body.

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Appreciation for the multi-functional character of pericytes is rapidly evolving, generating significant interest across many fields of research. Concurrently, there is indisputable evidence for the role played by mechanics and mechanobiology in many other cell types. These observations underscore the importance of understanding the potential involvement of pericyte mechanics and mechanobiology in health and disease (Eyckmans et al., 2011). While there are numerous reviews on pericyte biology (Armulik et al., 2011; Díaz-Flores et al., 2009; Gaceb et al., 2018; Rucker et al., 2000; Sweeney and Foldes, 2018; Sweeney et al., 2018), none focuses on the mechanical aspects. Here, we review and synthesize the literature from a variety of fields to extract important information about pericyte mechanics and mechanobiology. We begin by focusing on pericyte morphology and internal organization, as well as their physical interactions with other cells. We then turn our attention to contractile forces generated by pericytes, mechanical forces exerted on pericytes, and pericyte responses to these forces. Finally, we describe *in vitro* models for studying various aspects of pericyte function. Throughout the Review, we highlight key open questions and make the case for smarter engineered *in vitro* systems to address these questions.

Pericyte morphology and intracellular organization

The triad of pericyte morphology, activation and migration

While recent advances in 'omic' techniques promise to provide more definitive pericyte identification (Chasseigneaux et al., 2018; Vanlandewijck et al., 2018), the current approach is to use either a pericyte-specific combination of biomarkers or the morphological description of a protruding soma with slender processes (Armulik et al., 2011; Smyth et al., 2018). Pericytes exist in one of two states – quiescent or active – each with a characteristic cell shape and structure (Díaz-Flores et al., 2009).

Quiescent pericytes are encased within the vascular BM and have two distinct substructures – the soma, a protruding cell body within which the nucleus is housed, and slender (0.02–0.5 µm diameter) elongated processes (Bruns and Palade, 1968; Fujiwara and Uehara, 1984). Primary processes extend along the vessel axis, while secondary processes wrap around the vessel circumference. Quiescent pericytes are subdivided into three subtypes based on their morphology and their localization within the vascular tree: ensheathing pericytes (ePCs) on pre-capillaries, thin stranded pericytes (tsPCs) on capillaries, and stellate pericytes (sPCs) on post-capillaries (Fig. 1) (Attwell et al., 2016; Berthiaume et al., 2018b; Grant et al., 2019) (see also Box 1). In ePCs, the primary processes are short, while the secondary processes are wide and fully encircle the vessel. In tsPCs, the primary processes can be hundreds of micrometers long, whereas the secondary processes are short and only partially enwrap the vessel (Hill et al., 2015). In sPCs, this orthogonal organization of primary and secondary processes is lost, and the processes are distributed in a fractal-like branching pattern (Hill et al., 2015). In all subtypes, processes often colocalize with EC junctions regardless of the extent of vessel coverage, which is high for ePCs and sPCs, and low for tsPCs (Grant et al., 2019; Sims and Westfall, 1983).

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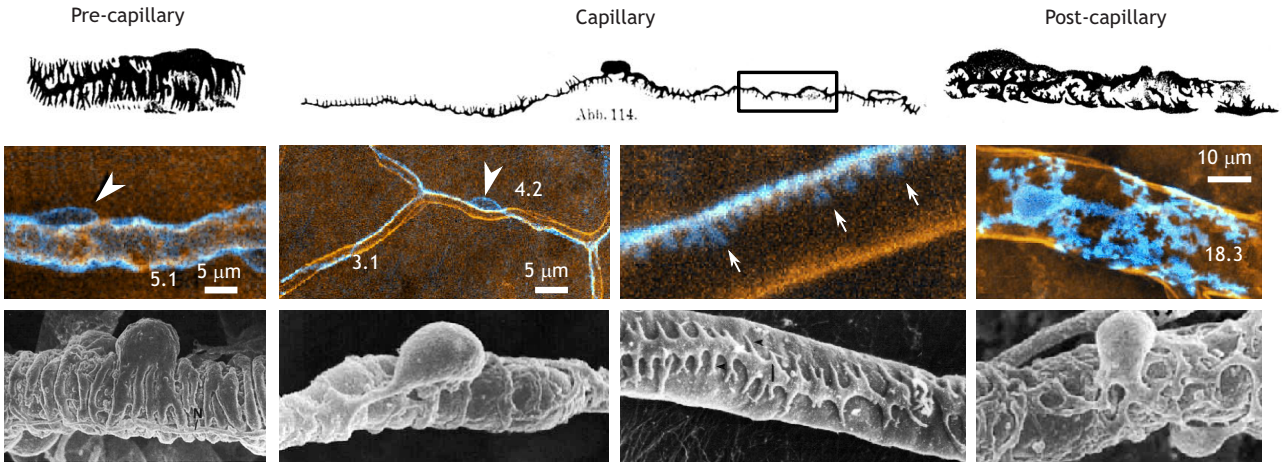

Fig. 1. Morphology of pericyte subtypes. Illustration of the three quiescent pericyte subtypes based on their morphology and location, using different techniques. Golgi-Kopf silver staining (top row; adapted from Zimmermann, 1923) and fluorescence imaging of pericytes (blue) in a transgenic mouse model [middle row; reprinted from Hill et al. (2015) with permission from Elsevier]. Here, arrowheads point to protruding somas and arrows in the close-up indicate short secondary processes. Numbers refer to vessel diameters in micrometers. The bottom row shows scanning electron microscopy image of the pericyte subtypes. The left, second from left and right-hand panels on the bottom row are reprinted from Fujiwara and Uehara (1984) with permission from John Wiley and Sons. The second from right panel on the bottom row is reprinted from Mazanet and Franzini-Armstrong (1982) with permission from Elsevier.

Pericyte activation is observed in developing and regenerating tissue. The active state is characterized by pericytes breaking out of the BM in what appears to be two distinct stages (Díaz-Flores et al., 2009; Payne et al., 2019). In the initial stage, existing processes retract and detach from the vessel wall, the soma assumes a triangular shape, a new process emanates from the pericyte body and the nucleus elongates orthogonally to the vessel axis. In the second stage, pericytes migrate into the parenchymal tissue. An example of pericyte activation is during angiogenesis, where pericyte detachment is necessary for EC migration and proliferation (Dore-Duffy and Cleary, 2011; Gonul et al., 2002; Hou et al., 2018; Payne et al., 2019; Pfister et al., 2008). Interestingly, active pericytes have been observed to be closely associated with ECs at the tips of angiogenic sprouts, sometimes with an antenna-like process pointing towards the target vessel (Errede et al., 2018; Payne et al., 2019; Stapor et al., 2014), suggesting that pericytes might participate in directional EC migration.

Although essential for a variety of functions (Bergers and Song, 2005; Stapor et al., 2014), pericyte activation is poorly understood. Several biochemical pathways appear to be involved (Díaz-Flores et al., 2009); however, data also point to a role for mechanics. For instance, excessive contraction can trigger pericyte activation during angiogenesis (Durham et al., 2014). Another example comes from early development where mural cell recruitment to the vessel wall, suggestive of return to quiescence, is concomitant with the onset of flow and the ensuing surge in hemodynamic stresses (Shen and McCloskey, 2017; Sweeney and Foldes, 2018).

An important distinction between quiescent and active pericytes is in their mode of migration. Quiescent pericytes exhibit 2D ‘crawling’ along the vessel wall, whereas the migration of active pericytes often takes the form of ‘escaping’, a 3D migration away from the vessel into the tissue. It is unclear how these two modes relate to the two well-characterized types of cellular migration, ameboid and mesenchymal (Yamada and Sixt, 2019).

Crawling pericytes are mostly seen during embryogenesis when mural cells colonize newly formed vessels. Crawling consists of two steps – a slow (hours) extension of the processes, frequently along EC junctions, followed by a swift (tens of minutes) translocation of the cell body (Fig. 2Ai) (Ando et al., 2016). Fibronectin, the preferred extracellular matrix (ECM) adhesion molecule of pericytes, is confined to the perivascular space and may be involved in crawling guidance (Grazioli et al., 2006). A similar two-step process is found in migratory neurons (Cooper, 2013; Nakazawa and Kengaku, 2020). Our more advanced understanding of neuronal migration promises to guide the design of future studies of pericyte 2D migration. In the adult vasculature, the first step of crawling appears to also maintain pericyte coverage of vessels after injury. For instance, after laser ablation of a tsPC, neighboring pericytes extend their processes to cover the denuded endothelium (Fig. 2B) (Berthiaume et al., 2018a). Interestingly, the soma is shown to stay immobile, contrary to what occurs during the second step of crawling.

Pericyte escaping, which is a reaction to stress, occurs when cells transition from the quiescent to the active state (Dore-Duffy et al., 2000; Gonul et al., 2002; Hou et al., 2018; Pfister et al., 2008). Pericytes retract their processes, digest the overlying BM, push their soma outward, extend a protrusion away from the vessel and migrate into the surrounding tissue (Fig. 2Aii). During certain scenarios, such as angiogenesis, both migration modes appear to coexist, with

Box 1. Pericyte continuum

Pericyte categorization is more nuanced than a classification in three subtypes and two states. Additional complexity stems from intermediate subtypes, such as the mesh pericytes and pre-capillary sphincters. Mesh pericytes have a high level of vessel coverage and encircling circumferential processes similar to ePCs, but their lack of SMA and their thinner processes resemble what is seen in tsPCs (Grant et al., 2019). Pre-capillary sphincters, which share attributes of SMCs, are also thought to belong to the pericyte family (Grubb et al., 2020).

Bridging pericytes, cells with processes reaching through the tissue toward a neighboring vessel, were described in the original drawings of Zimmermann (1923) and in later studies (Hartmann et al., 2021). A more recent study has also identified inter-capillary bridging cells as pericytes whose somas are not located in the vascular BM but in the parenchyma (Alarcon-Martinez et al., 2020; Corliss et al., 2020; Mendes-Jorge et al., 2012). These cells have the characteristics of the active state but are found in quiescent tissue, challenging the binary distinction between the two states. Dynamic observation of pericytes is therefore crucial to refine our current understanding of pericyte structure and function.

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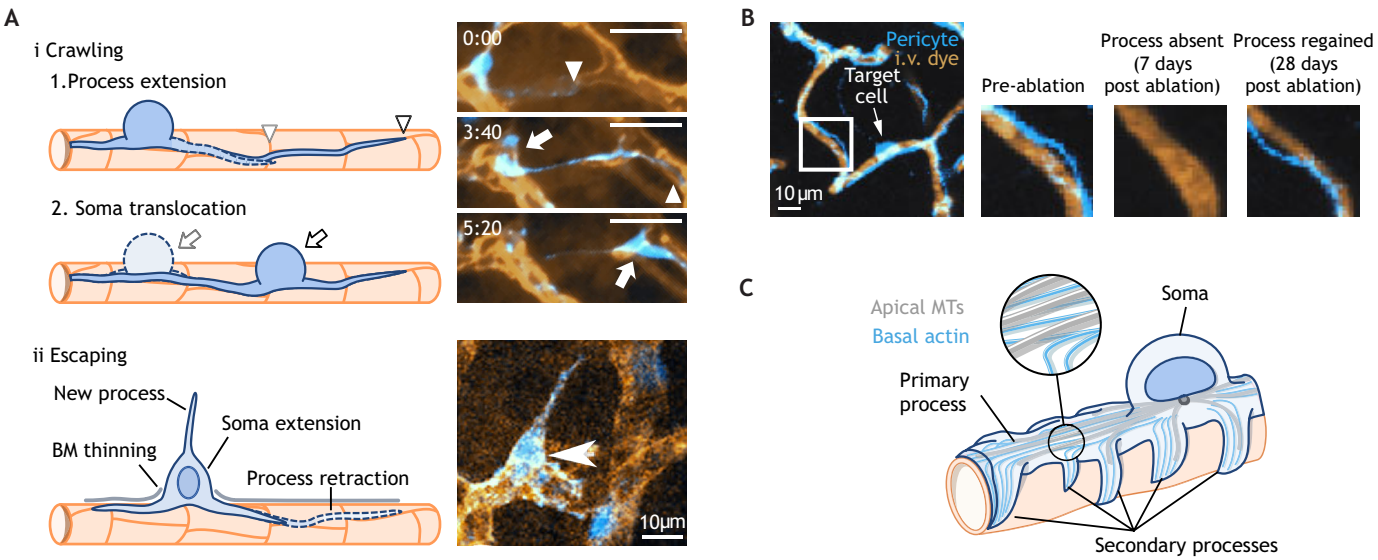

Fig. 2. Pericyte migration and intracellular organization. (A) Pericyte migration modes. (i) Two-step crawling of a mural cell in the zebrafish embryo with slow process (arrowheads) extension and swift soma (arrows) translocation. Adapted from Ando et al., 2016 with permission. Times in the images are given in hour:min after image in top row. Scale bars: 25 µm. (ii) Escape of a pericyte (arrowhead in image) in a mouse model after acoustic trauma. Reprinted with permission from Springer Nature, Journal of the Association for Research in Otolaryngology (Hou et al., 2018). (B) Targeted ablation of a pericyte results in dilation of the vessel after loss of the primary process. This is recovered after extension of a process from a neighboring pericyte (images on the right), illustrating the role of pericytes in mechanical strengthening of the vessel wall. Reprinted from Berthiaume et al. (2018a) with permission from Elsevier. (C) Schematic illustration of the pericyte cytoskeleton with apical microtubules predominant near the soma and basal actin predominant in the processes. Note the longitudinal and circumferential orientation, respectively, presumably to optimize the effectiveness of force transmission.

pericytes crawling around nascent tubes or escaping into the parenchyma. The interplay between mechanical forces and cell migration, which has been extensively studied in other cell types (Kurniawan et al., 2016), remains poorly characterized in pericytes and should attract significant attention in the coming years.

Pericyte intracellular organization and implications for force generation and transmission

The pericyte soma contains the bulk of the cytoplasm and most organelles, including the nucleus, whereas the processes consist primarily of cytoskeletal elements. In quiescent pericytes, the nucleus is discoid, close to the vessel wall and heterochromatic. Conversely, in active pericytes, the nucleus is round, protruding and euchromatic (Díaz-Flores et al., 2009). The nucleus in many cell types acts as a mechanosensor (Cho et al., 2017); therefore, the differences in nuclear shape and organization between quiescent and active pericytes may reflect different nuclear mechanical properties and might affect overall mechanosensing.

Pericytes have a bilayered cytoskeleton – microtubules and intermediate filaments are present primarily on the apical side within the cell body and the primary processes, whereas actin microfilaments localize mostly in the primary and secondary processes, where they form a dense continuous plaque on the basal surface (Fig. 2C) (Bruns and Palade, 1968; Forbes et al., 1977). The fibers are oriented axially in the primary processes and circumferentially in the secondary processes. Because cytoskeletal organization is a key factor in force sensing, generation and transmission (Gouget et al., 2016; Hwang and Barakat, 2012; Lebeux and Willemot, 1978), and because fiber orientation typically reflects the direction of the predominant forces, the circumferential orientation of pericyte secondary processes makes them compelling candidates for vessel constriction. Actin filaments in pericyte processes are anchored at various points along their lengths to ECs, forming adhesion plaques that are bound to the BM (Alliot et al., 1999), suggesting an important role for these filaments in force transmission. Whether microtubules and intermediate filaments play a role in pericyte force generation and transmission remains unknown.

The canonical model for cellular force generation involves molecular motors moving on tensile cytoskeletal cables. Both actin filaments and non-muscle myosin II, which are the principal constituents of force-generating actin stress fibers, are present in pericytes (Hamilton, 2010; Lebeux and Willemot, 1978; Tojkander et al., 2012; Vanlandewijck et al., 2018). The presence of α-smooth muscle cell actin (α-SMA, encoded by *ACTA2*), an actin isoform prominent in smooth muscle cells (SMCs) that amplifies stress fiber formation and is part of the cellular contractile machinery (Wang et al., 2006), varies among pericyte subtypes and is the subject of an ongoing debate (Attwell et al., 2016; Nehls and Drenckhahn, 1991). Most studies report the presence of α-SMA in ePCs and sPCs, with an expression level between that of ECs and SMCs. The results are more nuanced for tsPCs, where different reports paint divergent pictures (Attwell et al., 2016; Nehls and Drenckhahn, 1991). The advent of new imaging techniques, such as super-resolution microscopy and serial block face electron microscopy, should improve our knowledge of pericyte cytoskeletal organization in coming years (Calì et al., 2019; Hoffman et al., 2020; Harris et al., 2020).

Pericyte physical interactions

Physical anchoring to the BM

Pericytes interact physically with both the BM and neighboring ECs, as well as with other pericytes. The microvascular BM is formed by two ECM networks, one comprising laminin and the other collagen IV, bound together by perlecan (HSPG2) and nidogen (NID1 and NID2) (Leclech et al., 2020; Thomsen et al., 2017). In addition, fibronectin patches, in either circumferential crescent shapes or dots, punctuate the BM and are found exclusively between pericytes and ECs (Courtoy and Boyles, 1983). The higher

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fibronectin levels in developing tissues induce pericyte activation, whereas the higher laminin levels in mature tissues promote pericyte quiescence. These observations underscore the role of BM composition in regulating pericyte state (Risau and Lemmon, 1988).

In order for force transmission to occur, a physical connection between the pericyte cytoskeleton and the BM and/or ECs is necessary. Fibronectin patches in the BM colocalize with pericyte focal adhesions, thus linking to actin filaments in the processes. Notably, actin cables can bind tangentially to consecutive adhesion plaques shared with ECs (Courtoy and Boyles, 1983). These adhesion plaques are hypothesized to be anchoring points for pericytes and part of a physically continuous force transmission chain.

Pericyte–EC interactions

While most reviews have focused on the chemical pathways regulating pericyte–EC communication (Díaz-Flores et al., 2009; Gaceb et al., 2018; Kolinko et al., 2018; Sweeney and Foldes, 2018; Sweeney et al., 2016), there is mounting evidence that direct physical interactions are also centrally involved. To enable direct contact, pericyte secondary processes protrude through the endothelial BM to touch ECs, forming peg–socket invaginations, gap junctions and adherens junctions (Fig. 3).

Peg-and-socket invaginations are micron-sized structures, whereby a pericyte protrusion 'pokes' inside an EC (Fig. 3A) and potentially act as a hook that pulls on the EC (Braverman and Sibley, 1990; Caruso et al., 2009). Gap junctions are connexin assemblies that form transmembrane channels, allowing rapid cytosolic exchange between cells (Fig. 3B). Connexin 43 (also known as GJA1), which forms mechanosensitive hemichannels in ECs and astrocytes (Chen et al., 2018; Cherian et al., 2005; Islam and Steward, 2019), is also present in pericytes (Hirschi et al., 2003; Kolinko et al., 2018; Ivanova et al., 2019), but its role in force sensing has not been investigated. Pericyte–EC adherens junctions are composed of transmembrane N-cadherins that directly link the actin cytoskeleton in the two cell types (Fig. 3C) (Kruse et al., 2019). These various structures are primary candidate sites for force transmission between cells. Identifying the mechanotransduction pathways associated with the physical coupling of pericytes to ECs and the BM warrants further attention.

Pericyte–pericyte interactions

Pericytes do not appear to make stable physical contact with neighboring pericytes despite their close proximity. Instead, they form discontinuous layers with visible spaces between adjacent cells, reminiscent of a possible contact-avoidance mechanism (Attwell et al., 2016; Berthiaume et al., 2018b; Grant et al., 2019). Indeed, this pattern is a morphological marker often used to differentiate pericytes from SMCs. Pericytes are distributed regularly across the microvasculature (Kovacs-Oller et al., 2020); this fine control of the relative positioning of adjacent pericytes may enable transient physical contact in order to detect neighbors. In line with this hypothesis, brain capillary pericytes have been shown to constantly probe their surroundings by extending their processes and then retracting them back after touching other pericytes (Berthiaume et al., 2018a); this allows the pericyte network to remodel after injury. For instance, after laser ablation of a single pericyte, neighboring cells extend their primary processes to reestablish vessel coverage, but only until they meet another process (Berthiaume et al., 2018a; Hartmann et al., 2021). The nature and dynamics of the contact between the tips of each cell process remain unknown and certainly merit further study.

Forces by and on pericytes

Forces generated by pericytes – pericyte contractility

In his 1873 description of pericytes as a new type of mural cell, Rouget speculated that one of their functions was mediating blood

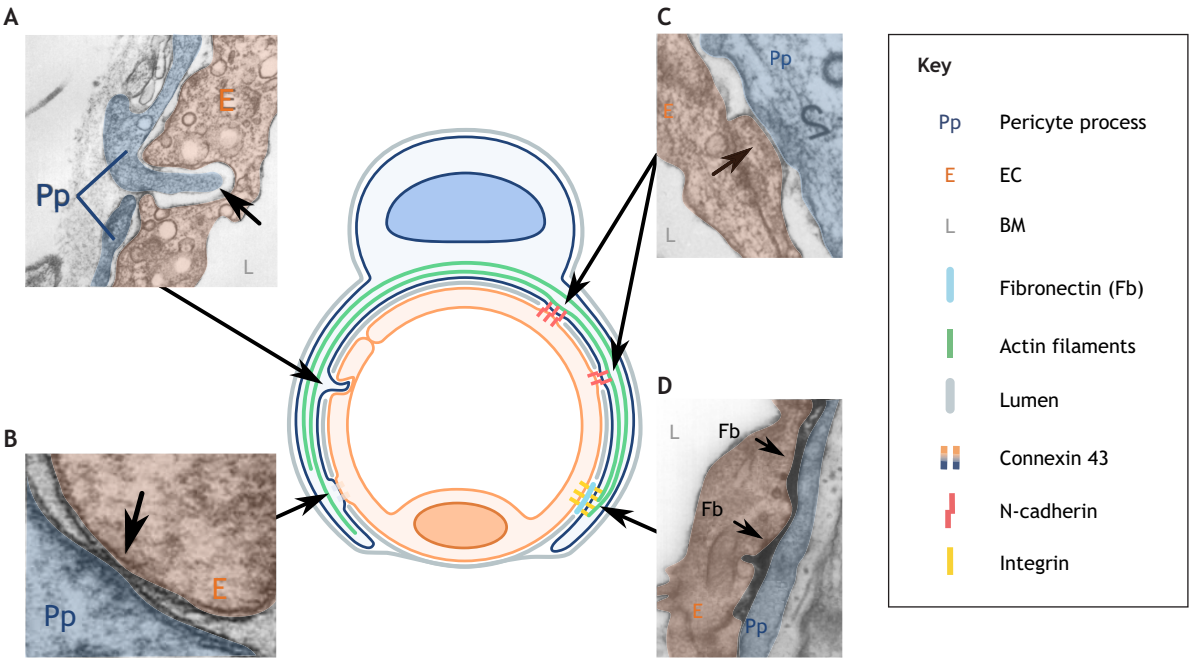

Fig. 3. Pericyte–EC interactions. Schematic illustration of the physical interactions between pericytes and ECs that are necessary for force transmission from pericyte to the vessel. (A) Peg and socket. Micron-scale invagination of the pericyte membrane into the EC. (B) Gap junctions. Direct apposition of both membranes, often containing connexin 43 channels. (C) Adherens junctions. Cell–cell contacts mediated through transmembrane proteins such as N-cadherins, which are linked to the cytoskeleton in each of the cells. (D) Adhesion plaques. Pericytes are linked to fibronectin patches in the BM through their integrins; this also provides an indirect link to ECs. Panel B republished with permission from Rockefeller University Press from Bruns and Palade (1968); permission conveyed through Copyright Clearance Center, Inc. Panels A, C and D are reprinted from Courtoy and Boyles (1983) with permission from Elsevier.

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flow control via their contraction. Since then, there has been an ongoing debate as to whether or not pericytes are truly contractile cells (Attwell et al., 2016; Fernandez-Klett et al., 2010; Hamilton, 2010; Hill et al., 2015). The controversy is in part attributable to difficulties in definitively identifying the different pericyte subtypes and the apparent differences in α-SMA levels among these subtypes, as mentioned above. Additionally, it is our belief that the dissension stems partly from a lack of consensus on the meaning of the term 'contractility'. If one goes by the simple definition of contractility as a 'reduction to smaller size', then pericytes are clearly contractile cells. We propose, however, that an additional consideration is the temporal dimension of contractility, as described next.

When thinking about cellular contractility, a key issue is the time scale under consideration (Hartmann et al., 2021). In the case of pericytes, three distinct contractility dynamics can be distinguished – quasi-static (time constant of days), slow (minutes), and fast (milliseconds). At the slowest time scale, pericytes modulate tension in the vessel wall (Berthiaume et al., 2018a,b). At the intermediate time scale, pericytes contract slowly, minutes after external stimulation, to regulate vessel diameter (Fernandez-Klett et al., 2010; Hartmann et al., 2021; Tilton et al., 1979). At the fast time scale, pericytes actively contract or dilate milliseconds after stimulation, rapidly modulating blood flow (Cai et al., 2018; Hall et al., 2014; Khennouf et al., 2018; Peppiatt et al., 2006; Rungta et al., 2018). A precise definition of contractility is therefore crucial, in particular for cerebral pericytes whose involvement in cerebral blood flow control is the subject of a heated debate (Bell et al., 2010; Brown et al., 2019; Grutzendler and Nedergaard, 2019; Lendahl et al., 2019).

Controlling blood flow is an essential function of mural cells. Half of the pericyte population is located at vascular branching points, ideal positions for flow control and redirection (Fernandez-Klett et al., 2010; Hartmann et al., 2015). Pericyte contraction and relaxation can generate up to a 20% change in vessel diameter (Hall et al., 2014; Cai et al., 2018). For fully developed steady flow in a cylindrical channel, the flow rate scales with channel diameter to the fourth power; therefore, even a small change in diameter has a drastic impact on flow rate. In one study, for instance, in a 4-µm capillary, a diameter increase smaller than the pixel resolution limit was sufficient to double red blood cell velocity (Rungta et al., 2018). As another example, the death of constricted tsPCs is thought to be a potential source of the no-reflow phenomenon following cerebral or cardiac ischemia, a direct demonstration of the importance of pericyte contractility in controlling blood flow (Cai et al., 2018; Hall et al., 2014; O'farrell et al., 2017).

How pericyte contraction enables blood flow regulation remains a subject of debate. One hypothesis is that pericyte contraction increases overall vessel wall stiffness, thereby limiting vessel dilation in response to increased blood pressure and simultaneously altering the EC mechanical environment (Berthiaume et al., 2018a; Durham et al., 2014; Lee et al., 2010; Rhodin, 1968; Rungta et al., 2018). The high concentration of pericytes in the vicinity of EC junctions suggests a protective role, possibly preventing deleterious junction opening due to stress concentrations generated by elevated blood pressure (Fig. 4Ai) (Shepro and Morel, 1993; Harris et al., 2020). A second hypothesis posits that dynamic pericyte contraction deforms underlying ECs. Pericytes can reduce vessel perimeter by pulling on ECs and can even occlude the lumen by physically buckling the underlying EC membrane (Fig. 4Aii) (Nahirney et al., 2016; Tilton et al., 1979). Processes that completely encircle the vessels can exert forces normal to the vessel surface, as suggested by visible indentation marks on ECs just below pericyte processes (Harris et al., 2020), in order to constrict the lumen (Fig. 4Aiii) (Murphy and Wagner, 1994). Furthermore, when acting on EC fenestrations in organs where higher rates of exchange between intra- and extra-vascular compartments are required, such as skin or lung, pericyte-mediated forces enlarge the openings to facilitate fluid exchange (Fig. 4Aiv) (Imayama and Urabe, 1984).

Pericyte contractility appears to vary among subtypes. While it is widely accepted that ePCs actively contract, this remains controversial for tsPCs (Berthiaume et al., 2018a; Hall et al., 2014; Hartmann et al., 2021; Rungta et al., 2018). The contractility of sPCs remains unexplored, but their lack of orthogonal organization casts doubt on their capacity for dynamic contraction. An intriguing recent study reported that pericytes at vascular junctions can exhibit compartmentalized contraction, whereby they are able to selectively contract the processes around only one of the downstream daughter vessels, and they thus might act as flow switches (Gonzales et al., 2020). If validated, the notion of compartmentalized contraction has the potential to transform our understanding of pericyte contractility.

Forces exerted on pericytes

In vivo, pericytes are subjected to a complex and dynamic mechanical environment. Pericytes experience solid contact stresses due to their physical interactions with the BM and with ECs, and they are also subjected to hydrodynamic pressure and shear stresses from fluid flow. These forces arise from both transmural and interstitial flows (Fig. 4B). The transmural pressure

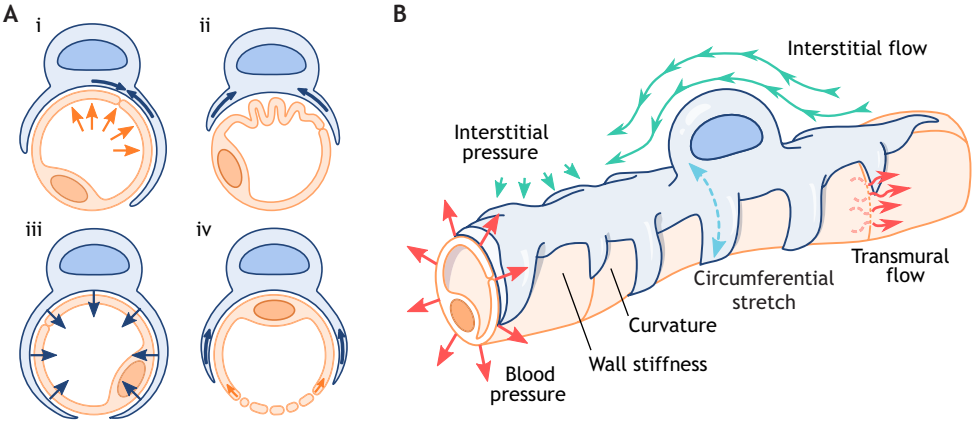

Fig. 4. Forces by and on pericytes. (A) Schematic illustration of the effects of pericyte contraction. (i) Strengthening of EC junctions to resist luminal pressure. (ii) Tangential pulling, inducing narrowing and buckling of the underlying EC. (iii) Vessel constriction through normal forces. (iv) Opening of fenestrations through tangential pulling. (B) Pericytes are subjected to many mechanical cues in their environment, including substrate–contact forces and topographic cues (orange), circumferential stretch (blue), as well as hemodynamic pressure and shear forces due to transmural (red) and interstitial (green) flow.

difference leads to fluid crossing the vascular wall through endothelial cell–cell junctions and thus impinging on pericytes and flowing around them, generating pressure and shear forces. Although the resulting shear stresses are unknown, a useful point of reference is the shear stress on the surface of SMCs due to transmural flow in medium and large arteries, which has been estimated to be ∼0.01–0.1 Pa (Wang and Tarbell, 1995). Interestingly, because of differences in their localization, the different pericyte subtypes are expected to experience different magnitudes of these hydrodynamic stresses as the transmural pressure difference decreases from arterioles to venules. Interstitial shear and pressure forces are generated by the movement of fluid within the tissue surrounding the vessel (Chary and Jain, 1989). Although the fluid velocity within tissue is relatively low, ∼1 µm/s, the corresponding shear forces can be significant because of the relatively low tissue porosity (Pedersen et al., 2010; Polacheck et al., 2011). The interstitial pressure forces to which pericytes are subjected are expected to be different for each subtype because of the differences in localization; the pressure is estimated at ∼50 mmHg for ePCs, ∼30 mmHg for tsPCs and ∼15 mmHg for sPCs (Parazynski et al., 1993; Slaaf et al., 1987).

Pericytes are mostly found on tortuous microvessels with a diameter smaller than 20 µm; thus, the cell-scale non-zero Gaussian curvature is expected to lead to significant bending stresses. Pericytes are also frequently located at branch points, where the available surface area is relatively large and the curvature small, suggestive of a mechanism of energy minimization. The transmural pressure difference in a microvessel subjects pericytes to oscillatory circumferential (hoop) stresses. Although the dominant view is that blood pulsatility gets dampened before it reaches the microvasculature (O'Rourke and Safar, 2005), recent measurements have challenged this view. Indeed, significant pressure and velocity oscillations have been reported in capillaries down to 4 µm in diameter (Gu et al., 2018; Gurov et al., 2018; Koutsiaris, 2016), and 40-µm-diameter mouse brain arterioles have been shown to exhibit 10% strain at the heartbeat frequency, demonstrating that the pulse penetrates deep into the microvasculature (Atry et al., 2018).

Forces on cells lead to deformations, but the extent of deformation depends on the mechanical properties of the cells. Atomic force microscopy (AFM) measurements on cells cultured on 2D rigid surfaces have reported a Young's modulus of ∼10 kPa for pericytes, ∼3 kPa for ECs and ∼2 MPa for the vascular BM (Candiello et al., 2007; Lee et al., 2010; Vargas-Pinto et al., 2013). These values, combined with the thickness of each of the structures, provide a measure of their relative stiffnesses and hence their load-bearing capacity. This information is useful for assessing the validity of certain hypotheses. For instance, a hypothesized function of pericytes is to provide structural support for the microvascular wall. In support of this notion, the loss of pericytes leads to a weakened vascular wall and microaneurysms in mice, whereas the presence of mural cells is correlated with narrower and, possibly, less-compliant vessels (Lindahl et al., 1997; Stratman and Davis, 2012). In order for the structural support hypothesis to be plausible, the contribution of pericytes to the overall vessel wall stiffness must be at least comparable to that of ECs and the BM. Thus, determination of pericyte mechanical properties is critical for validating the pericyte structural support hypothesis.

All measurements of pericyte mechanical properties to date have been performed on cells *in vitro*, and thus with non-physiological morphology and internal structure. There is a need for establishing how the stiffness of pericytes *in vivo* compares to that *in vitro*, and whether different pericyte subtypes and states differ in their mechanical properties. Intracellular organelles and cytoskeletal elements are major contributors to cellular mechanics. The soma, with its large cytosol and nucleus, is likely to be relatively soft and viscous, whereas processes, with their predominant actin fibers, are expected to be stiffer. Hence, different parts of a single pericyte are likely to have considerably different mechanical properties, as has been shown in other cell types (Deville and Cordes, 2019; Gabriele, 2014).

Pericyte mechanosensing and mechanotransduction

Although direct demonstrations remain sparse, there is mounting evidence that pericytes, like virtually all other cell types, sense and respond to the mechanical forces to which they are subjected. A particular difficulty lies in establishing whether or not the mechanical stimulus is causative of the observed biological response. For instance, in muscle and skin, pericyte distribution is higher in the lower portions of the body where hydrostatic pressure is more elevated (Sims et al., 1994). The brain and kidney, whose vasculature has a low hydrodynamic resistance, and is therefore exposed to higher pressure pulsatility, exhibit greater numbers of pericytes (O'Rourke and Safar, 2005). In the developing embryo, the onset of hemodynamic forces is concomitant with mural cell recruitment (Shen and McCloskey, 2017). Finally, inflammation-induced increases in transmural flow (and thus endothelial leakage) lead to pericyte reorganization and increased coverage of endothelial junctions (Sims, 2000).

Unraveling the pericyte responses to each of the biophysical cues described above is a challenge because most cues are coupled. For instance, the lungs of hypertensive adults exhibit a two-fold increase in the number of pericytes compared to what is seen in healthy individuals (O'Rourke and Safar, 2009). However, hypertension implies not only elevated pressure but also an elevated pulse pressure amplitude, larger tissue strain and higher transmural flow, all of which might trigger the increased pericyte density (Ricard et al., 2014). Thus, *in vitro* models in which individual mechanical parameters can be independently controlled and the overall environment simplified to incorporate only the components of interest are a promising way forward as discussed henceforth.

In vitro models

In vitro systems are highly simplified versions of the *in vivo* environment in which individual parameters of interest can be manipulated in a more controlled manner. Engineering a physiologically relevant environment and validating cellular behavior against known *in vivo* data whenever possible are essential for ensuring the pertinence of *in vitro* results. Here, we review *in vitro* platforms used for the study of various aspects of pericyte structure and function, and discuss their current limitations and future opportunities.

Controlling pericyte structure

Pericytes in *in vitro* systems typically suffer from a loss of phenotype, casting doubt on the true identity of the 'pericytes' used *in vitro*. For example, pericytes cultured on stiff 2D substrates exhibit a flat shape, large area, increased levels of α-SMA and prominent actin stress fibers (Fig. 5A) (Boado and Pardridge, 1994; Dore-Duffy and Cleary, 2011; Durham et al., 2014). These features are absent *in vivo*, but it remains unclear whether these changes are accompanied by functional anomalies (Rustenhoven et al., 2018). Recent experimental systems and protocols have reported a more physiological structure. For instance, pericytes obtained with new differentiation protocols from induced pluripotent stem cells (iPSCs) show lower levels of SMA (Stebbins et al., 2019), while 3D cultures of pericytes in soft gels allow them to adopt a more

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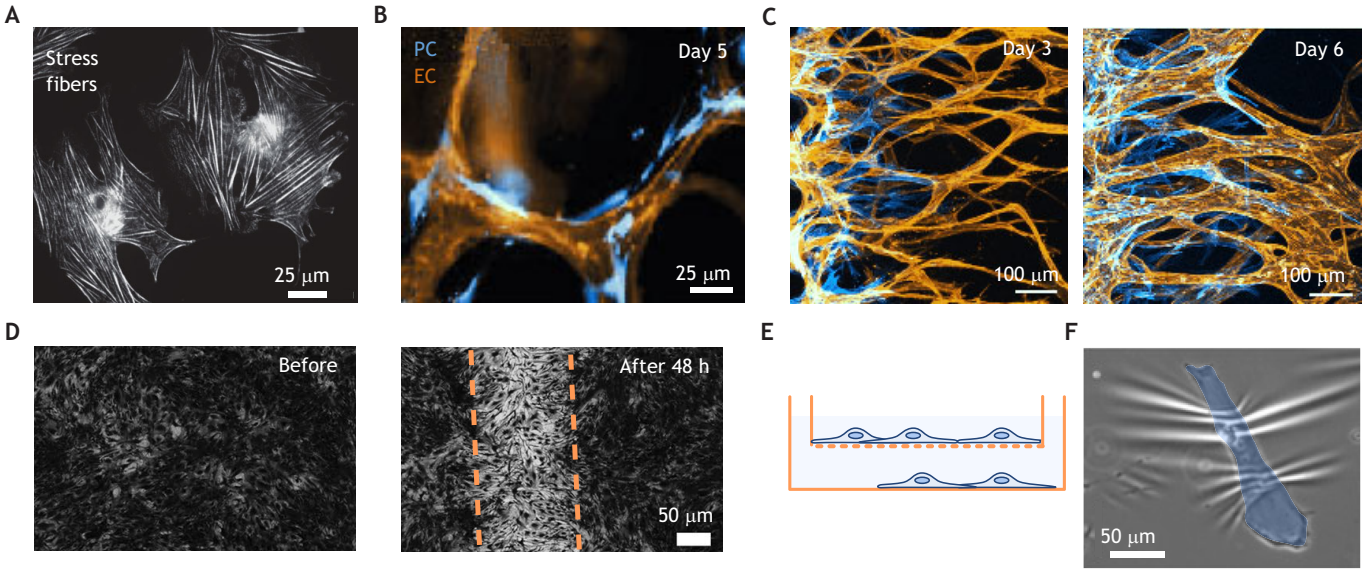

Fig. 5. *In vitro* systems for studying pericytes. (A) Culture on 2D stiff substrates. Here, pericytes assume a flat spread morphology, exhibiting prominent stress fibers. Adapted with permission from Durham et al. (2014). (B) Tubulogenesis (spontaneous formation of vessels by cells suspended in a gel). Pericytes surround thin EC tubes. Adapted with permission from Stratman and Davis (2012). (C) Angiogenesis (sprouting assay in gels). Pericytes migrate along EC tubes with an elongated shape. Adapted from Kim et al. (2015), where it was published under a CC-BY 4.0 license. (D) Wound healing. Migration of pericytes after scratch wounding of a confluent layer. Adapted from Rustenhoven et al. (2018), where it was published under a CC-BY 4.0 license.. (E) Transwell chambers. Migration of pericytes through a synthetic porous membrane. (F) Contractility assays. Wrinkling of a flat soft thin membrane by pericytes. Reprinted from Pellowe et al. (2019) with permission from John Wiley and Sons.

physiological morphology with a defined soma and short protrusions (Alimperti et al., 2017; Kim et al., 2015).

Investigating BM interactions

The vascular BM is composed of several components whose relative abundance changes during development, angiogenesis or disease (Leclech et al., 2020). *In vitro* platforms allow investigation of the effect of individual BM components on pericyte structure and function. Indeed, such studies have shown that pericytes adhere less avidly to laminin-1 or heparan sulfate proteoglycan (HSPG) than to collagen IV or fibronectin, all components of the native BM (Tigges et al., 2013). Furthermore, fibronectin induces higher rates of pericyte proliferation and migration (Tigges et al., 2013), an observation that may explain the increased fibronectin levels observed during angiogenesis and development, where pericyte activation and migration are crucial. Interestingly, the mechano-response to substrate composition and stiffness is defective in pericytes isolated from end-stage ischemic hearts (Rolle et al., 2020), illustrating the crucial yet poorly understood role of pericyte mechanobiology in diseases.

A recent study that used an engineered substrate has further underscored pericyte preference for fibronectin; here, cells formed focal adhesions exclusively on fibronectin dots that had been positioned amidst a laminin-coated surface, thereby reorganizing their entire cytoskeleton (Iendaltseva et al., 2020). Fibronectin distribution *in vivo* follows a similar dot pattern, being located at adhesion plaques in the BM between pericytes and ECs. This study highlights how common microfabrication techniques and state-of-the-art *in vitro* systems can greatly enhance our understanding of pericyte mechanobiology.

Mimicking cell–cell interactions

Despite the importance of pericyte–EC interactions for many pericyte functions, replicating the correct cell–cell organization *in vitro* is an ongoing challenge. Pericyte–EC co-culture experiments are usually performed using either flat 2D surfaces or 3D designs aimed at mimicking tubulogenesis (Fig. 5A,B) (Gökçinar-Yagci et al., 2015). In 2D co-cultures, ECs form discontinuous patches and pericytes populate the spaces in between, exhibiting limited intercellular contacts (Durham et al., 2014). 3D tubulogenesis models have been used to assess the supporting role of pericytes in EC tube formation by using a soft hydrogel, often Matrigel or collagen, allowing increased intercellular contact (Fig. 5B) (Stratman and Davis, 2012; Stratman et al., 2011; Zhao et al., 2018).

Other platforms have also been employed to investigate pericyte–EC interactions. An example is the Transwell system, where each cell type is cultured on one side of a synthetic membrane with a known pore size and whose thickness is ∼10 times that of the BM it is intended to mimic. This system restricts the area of cell–cell contact to discontinuous spots (Fig. 5E) (Alimperti et al., 2017). Another example are 3D microvessels, often termed microvessels-on-chip, which typically are large cylindrical channels fabricated inside a soft hydrogel, most commonly collagen or fibrin. Here, ECs line the lumen, while pericytes are embedded in the gel in close proximity to the ECs, thereby leading to a more physiological morphology (Fig. 5B,C) (Hu et al., 2017; Kim et al., 2015; Zheng et al., 2012).

Quantifying contractility

Pericyte contractility can be studied *in vitro* by examining pericyte-induced substrate deformations. The most common technique in 2D is the wrinkling assay, where cells plated on a thin layer of poly-dimethyl-siloxane (PDMS) wrinkle the substrate when they contract (Fig. 5F) (Kelley et al., 1987; Kotecki et al., 2010; Kutcher et al., 2007; Lauridsen and Gonzalez, 2017). Using this technique, the forces generated by individual pericytes have been estimated to be ∼1.5 μN (Lee et al., 2010). As wrinkles can be visualized using polarized light, this technique allows real-time monitoring of force, thereby providing an opportunity to elucidate the contractility dynamics of pericytes in real time, even though the technique has

not yet been exploited for this purpose. In 3D, pericytes have been seeded in soft gels, and pericyte contraction has been demonstrated through the compaction of the gel (Kelley et al., 1987; Oishi et al., 2007). A drawback of this approach, however, is that it does not allow quantification of the force. Traction force microscopy, both in 2D and 3D, promises to generate quantitative, dynamic and robust force measurements (Iendaltseva et al., 2020; Schwarz and Soiné, 2015).

Identifying how pericyte contractility restructures the microvascular wall is a major open question and requires spatially and dynamically resolved experiments of pericytes that exhibit an *in vivo*-like morphology. To this end, a recent study has shown that pericyte contraction in 2D culture increases intercellular gaps between ECs and facilitates neutrophil transmigration (Pellowe et al., 2019), underscoring the thus far mostly untapped potential of *in vitro* platforms.

Investigating migration

In vitro platforms also provide controllable environments with high spatial and temporal resolution, two essential attributes to unravel the mechanisms underlying pericyte migration (Kurniawan et al., 2016). The most common approach for studying pericyte migration is live-cell tracking on hard flat surfaces, such as in wound-healing scratch assays (Fig. 5D) (Grazioli et al., 2006; Zhao et al., 2018). Another common technique is the Transwell system alluded to above (Fig. 5E). A limitation of the Transwell system, however, is that cells cannot be imaged during the migration (Casey et al., 2015).

3D gel cultures are more realistic systems that allow cells to adopt a native-like morphology. An example of this is the first *in vitro* observation of vascular guidance, where pericytes were shown to migrate in tunnels carved by ECs, which mimics crawling and their longitudinal migration in the perivascular space (Stratman et al., 2016). Organ-on-chip setups with high-resolution time-lapse imaging using live dyes have also been used to study pericyte migration during angiogenesis, and have demonstrated that pericytes migrate along the nascent sprouts (Kim et al., 2015).

Studying mechanics and mechanobiology

The field of pericyte mechanics and mechanobiology remains in its infancy. For instance, quantitative characterization of pericyte mechanical properties is lacking. AFM measurements in combination with the 2D wrinkling assay have revealed regional variations in elasticity within the same pericyte, as the Young's modulus was ∼15 kPa in parts of the cell above the wrinkled areas but only ∼5 kPa elsewhere (Lee et al., 2010). Extending such measurements of local elasticity to pericytes that possess a more physiological pericyte morphology would allow exploration of potential differences in mechanical properties between the soma and the processes as well as the impact of pericyte activation on these properties.

Although pericytes experience different mechanical stimuli *in vivo* as already discussed, studies investigating the effect of mechanical forces on pericytes are scarce. Thus far, only two studies have investigated the effect of fluid mechanical shear stress on pericytes and have shown that a 'low' shear stress of 0.4 Pa induces alignment in the flow direction (Grazioli et al., 2006) whereas a 'high' shear stress of 3 Pa leads to alignment orthogonal to the flow (Schrimpf et al., 2017). The mechanistic basis of these seemingly divergent results remains to be established. Additionally, pericytes under shear flow and in contact with ECs prevent EC-mediated matrix degradation, suggesting a possible mechanobiological mechanism for maintaining vascular stability. Uniaxial cyclic stretch (10% at 1 Hz) reduces pericyte proliferation, increases apoptosis and induces the formation of thick stress fibers perpendicular to the stretch direction (Beltramo et al., 2006; Suzuma et al., 2007). Finally, substrate rigidity appears to modulate pericyte α-SMA levels (Iendaltseva et al., 2020), suggesting a mechanobiological link to contractility regulation. These intriguing results constitute an appetizer in the extensive menu of pericyte mechanobiological questions that can potentially be addressed using state-of-the-art *in vitro* approaches.

Conclusions

Although pericytes participate in a wealth of biological processes, often involving physical cues, little is known about pericyte mechanics and mechanobiology. Recent years have witnessed a tremendous rise in the interest of the scientific community in pericytes as key players in neo-vascularization and the development of various pathologies, most notably cancer and neurovascular diseases. A better understanding of pericyte mechanics and mechanobiology will provide ample opportunities in these important biomedical areas. We believe that *in vitro* models are a promising path forward to help establish the causative role of mechanical factors in regulating pericyte function and dysfunction. We hope that, in the future, biologists will consider the mechanical world of pericytes, and biophysicists will focus upon this new field, bringing answers to many elusive and fundamental questions.

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Competing interests

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