Review

Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche

L. Díaz-Flores¹, R. Gutiérrez¹, J.F. Madrid², H. Varela¹, F. Valladares¹,4, E. Acosta², P. Martín-Vasallo² and L. Díaz-Flores Jr¹

¹Department of Anatomy, Pathology, Histology and Radiology, Faculty of Medicine, University of La Laguna, Tenerife, Spain, ²Developmental Biology Laboratory, Department of Biochemistry and Molecular Biology, University of La Laguna, Tenerife, Spain, ³Department of Cell Biology and Histology, Faculty of Medicine, University of Murcia, Murcia, Spain and ⁴CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain

Summary. We review the morphofunctional characteristics of pericytes and report our observations. After a brief historical background, we consider the following aspects of pericytes: A) Origin in embryonic vasculogenesis (mesenchymal stem cells, neurocrest and other possible sources) and in embryonic and postnatal life angiogenesis (pre-existing pericytes, fibroblast/myofibroblasts and circulating progenitor cells). B) Location in pericytic microvasculature and in the other blood vessels (including transitional cell forms and absence in lymphatic vessels), incidence (differences depending on species, topographical location, and type and stage of vessels) and distribution (specific polarities) in blood vessels. C) Morphology (cell body, and longitudinal and circumferential cytoplasmic processes), structure (nucleus, cytoplasmic organelles and distribution of microtubules, intermediate filaments and microfilaments) and surface (caveolae system). D) Basement membrane disposition, formation, components and functions. E) Contacts with endothelial cells (ECs) (peg and socket arrangements, adherent junctions and gap junctions) and with basal membrane (adhesion plaques). F) Molecular expression (pericyte marker identification). G) Functions, such as vessel stabilization, regulation of vascular tone and maintenance of local and tissue homeostasis (contractile capacity and vessel permeability regulation), matrix protein synthesis, macrophage-like properties, immunological defense, intervention in coagulation, participation in mechanisms that regulate the quiescent and angiogenic stages of blood vessels (including the behaviour of pericytes during sprouting angiogenesis and intussusceptive vascular growth, as well as pericyte interactions with endothelium and other cells, and with extracellular matrix) and plasticity, as progenitor cells with great mesenchymal potential, originating other pericytes, fibroblast/myofibroblasts, preadipocytes, chondroblasts, osteoblasts, odontoblasts, vascular smooth muscle and myointimal cells. This mesenchymal capacity is seen in a broad section on the perivascular mesenchymal cell niche hypothesis and in the concept of pericyte and EC “marriage and divorce”. H) Peculiar pericyte types, such as hepatic stellate cells (Ito cells), bone marrow reticular cells and mesangial cells. I) Involvement in pathological processes, such as repair through granulation tissue, pericyte-derived tumors, tumor angiogenesis and tumoral cell metastasis, diabetic microangiopathy, fibrosis, atherosclerosis and calcific vasculopathy, lymphedema distichiasis, chronic venous insufficiency, pulmonary hypertension, Alzheimer disease and multiple sclerosis. J) Clinical and therapeutic implications (de-stabilization of vessels or formation of a stable vasculature).

Key words: Pericytes, Mesenchymal cells, Angiogenesis, Stem cell niche

Introduction

Traditionally, pericytes are defined as extensively branched cells located in nonmuscular microvessels, capillaries and postcapillary venules (Majno, 1965), embedded within the microvascular basement membrane, and incompletely enveloping the endothelial cells (EC) with which they establish specific and focal contacts.

As we shall see below, the concept of pericytes has been currently extended in relation to origin, location (presence in other kinds of vessels and variations in
some organs), heterogeneity (pericytes are a heterogeneous population and the use of this term has been proposed to include all cells collectively in a perivascular location with similar morphology: pericytes and pericyte-like cells), polymorphism, diverse characteristics, variable expression of markers, presence of mural cells with properties intermediate between pericytes and vascular smooth muscle cells (VSMC) (unclear whether they represent phenotypic variants of the same lineage), interactions and interdependence of pericytes and EC, multifunctionality, participation in angiogenesis and repair, mesenchymal potentiality and their role in the perivascular mesenchymal stem cell niche, repercussions in pathology, and clinical and therapeutic implications.

Previous articles have reviewed different morphologic and functional characteristics of pericytes, highlighting increasing interest in this area (Sims, 1986, 1991, 2000; Díaz-Flores et al., 1991a; Tilton, 1991; Nehls and Drenkhahn, 1993; Shepro and Morel, 1993; Schor et al., 1995; Hirschi and D’Amore, 1996; Allt and Nehls and Drenkhahn, 2001; Bergers and Song, 2005; Hall, 2006). A new review, combining the classical and current contributions, is advisable, given the numerous contributions intensifying the role of pericytes as adult mesenchymal stem cells located in a particular niche, the recent data on their identification, regulation, interactions and plasticity and their role in different pathological processes, as well as their clinical and therapeutic implications.

Historical background

Pericytes were described by Charles Rouget (1873) with the term adventitial cells, owing their current denomination to Zimmermann, (1923). Previously, Eberth (1871) had noted their presence. Numerous terms have been used for these cells, including pericytes, adventitial cells, Rouget cells, intramembranous pericapillary cells, mural cells, pericapillary cells, periendothelial cells, perivascular cells, undifferentiated cells and deep cells (Rouget, 1873; Krogh, 1919, 1929; Zimmermann, 1923; Farquhar and Hartmann, 1956; Battig and Low, 1961; Cogan et al., 1961; Kuwabara and Cogan, 1963; Ashton and De Oliveira, 1966). Silver impregnation techniques showed pericyte morphology (Zimmerman, 1923; Plenk, 1928, Stensaas, 1975), transmission electron microscopy their ultrastructure (Movat and Fernando, 1964; Bruns and Palade, 1968; Rhodin, 1968; Crocker et al., 1970; Simionescu et al., 1974; Weibel, 1974; Forbes et al., 1977; Lebeux and Willemot, 1978; Allsopp and Gamble, 1979; Tilton et al., 1979a; Rhodin and Fujita, 1989; Hasan and Glee, 1990), and scanning microscopy their precise form and arrangement (Murakami et al., 1979; Maggioni et al., 1989; Shimada et al., 1992). The initial morphologic studies guided functional interpretations, while pericyte culture and EC/pericyte coculture procedures provided important data about the function of pericytes (see Pericyte functions). Morphofunctional observations during experimental and pathological neovascularization have enabled the study of pericyte behaviour in sprouting and intussusceptive angiogenesis, suggesting pericyte participation in the regulation of these processes (see Behaviour of pericytes during angiogenesis). Recently, the use of mice with mutations of vascular regulatory genes encoding ligands or receptors (growth factors, receptors, signalling molecules), as well as of antibody blocking and gain-of-function studies, have been instrumental in defining the regulatory mechanisms in which the pericytes participate (see Pericyte interaction with endothelium and other cells, and with extracellular matrix).

Origin

In the embryo, vessel development occurs by vasculogenesis (de novo vessel formation) and angiogenesis (neoformation of new blood vessels from the pre-existing microvasculature). In adult life the vascularization is formed by angiogenesis. In vasculogenesis, pericytes develop from mesenchymal stem cells (mesodermal origin) and from the neuro crest, depending on their location. Thus, in most vessels, pericytes originate from mesenchymal stem cells, while in the CNS and cardiac tract vessels, they can arise from the neural crest (Bergwerff et al., 1998; Creazzo et al., 1998; Yamashita et al., 2000; Etchevers et al., 2002; Ema et al., 2003). TGF-β1 drives the differentiation of progenitor cells (Bergwerff et al., 1998; Creazzo et al., 1998) into PDGFR-β positive precursors of pericytes, which are attracted by PDGF-β secreting EC (Creazzo et al., 1998; Hellstrom et al., 1999). Pericytes and EC probably share a common progenitor cell during vasculogenesis. Indeed, mesodermal FLK1+ angioblasts may originate pericytes or EC depending on platelet-derived growth factor-β (PDGFB) or vascular endothelial growth factor (VEGF) stimulation, respectively (Yamashita et al., 2000; Carmeliet et al., 2001). In addition, the rare possibility of embryonic EC transdifferentiation into pericytes may be considered (DeRuiter et al., 1997). Likewise, mesothelium may undergo epithelial-mesenchymal transition and contribute to the vascular mural cells and mesenchyma in the lung (Que et al., 2008) and myocardium (Mikawa and Gourdie, 1996; Dettman et al., 1998).

In embryonic and postnatal life angiogenesis, the origin of pericytes remains relatively unclear. Indeed, pericytes may be considered both as progenitors (see below) and/or as descendent cells. In this way, pericytes may develop from tissue-derived stem cells (themselves or fibroblast/myofibroblasts) and/or from peripheral blood pluripotent stem cells (circulating progenitor cells) (similar to that which occurs in vasculogenesis). As outlined above, among the local putative progenitor stem cells are the pericytes themselves and the fibroblast/myofibroblasts. Both possibilities are complementary, since the myofibroblasts share many features in common with the pericytes (Hall, 2006) and, during angiogenesis, the pericytes of the pre-existing
Pericytes (pericyte-like cells), location, incidence and distribution in blood vessels

Pericytes and pericyte-like cells, present in nearly all vertebrate tissues, are located on the abluminal side of the endothelial cells in the pericytic microvasculature (capillaries and postcapillary venules), both in continuous and in fenestrated microvessels (Figs. 1A-C). Besides, pericytes are present in pre-capillary arterioles and collecting venules (Sima et al., 1985; Sims, 1986, 1991; Allt and Lawrenson, 2001), although there is a gradual transition (a continuum of phenotypes) between pericytes and smooth muscle cells in both terminal arterioles and venules (Díaz-Flores et al., 1991a) (Fig. 1D). According to their location, three types of pericytes were classically established: precapillary, capillary and postcapillary (Zimmermann, 1923). A continuous subendothelial network formed by pericyte-like cells in large human vessels has also been suggested (Shchelkunov, 1935, 1977; Andreeva et al., 1998), corresponding to the intimal fibroblasts of Langhans (Langhans, 1866), mesenchymal reserve cells of Maximov (Maximov, 1927), pericytes vascular cambium of Shchelkunov (Shchelkunov, 1977) and the modified smooth muscle cell of other authors. In this way, using 3G5 antibody, pericyte-like cells were identified in the intimal layer, predominantly in the subendothelium, in the outer layer of the media and in the vasa-vasora of large, medium and small arteries, as well as in the subendothelium, media (both in the inner and outer layers) and vasa-vasora of the veins (Andreeva et al., 1998). Furthermore, in these vessels it was demonstrated that subendothelial-like cells form a network contacting with their processes (Andreeva et al., 1998).

In general, pericytes have a close spatial relation to ECs, and a single pericyte covers several endothelial cells incompletely (partial pericycle coverage of ECs) (Allt and Lawrenson, 2001). The number of pericytes (relative frequency of pericytes to endothelial cells) and the vascular pericycle coverage show substantial differences depending on species, topographical location, type of vessel and stage. Thus, the pericyte to endothelial cell ratio is greater in human than in rat retina (1:1 and 1:3, respectively – Cogan and Kuwabara, 1967; Tilton et al., 1985), in retina than in skeletal muscle (1:1 and 1:100, respectively – Shepro and Morel, 1967; Tilton et al., 1985), in post-capillary venules than capillaries (Rhodin, 1968; Simionescu et al., 1975), in quiescent than in vessels stimulated for angiogenesis (Díaz-Flores et al., 1991a, 1992a; Nehls and Drenckhahn, 1993) and in quiescent than in hemangiomas with EC proliferation (Feldman et al., 1978; Wakui et al., 1997). Vessel coverage by pericytes is greater in tissues with the slowest EC turnover (Tilton et al., 1985; Wakui et al., 1997). The distribution of pericytes around microvessels exhibits specific polarities according to the regions of oxygen transfer, filtration and nutrient transport (Armulik et al., 2005). Thus, pericytes tend to be concentrated in the non-gas exchange regions - Imayama and Urabe, 1984) (e.g. pericytes are located distally from the zone of oxygen transfer to root and cone in the human eye choriocapillaries - Matsusaka, 1969 - and in the dermal side of the subepidermal capillaries - Imayama and Urabe, 1984). In general, pericytes are positioned around endothelial cell junctions (Sims and Westfall, 1983; Sims, 1991), forming umbrella-like...
Fig. 1. Pericyte location, distribution and characteristics. A-C. Pericytes (P) located in the abluminal side of endothelial cells (EC) in a continuous (A) and in two fenestrated vessels (B, C-arrows). D. A transitional cell (TC) between pericytes and smooth muscle cells (SMC). E, F. Pericyte location around interendothelial gaps (arrows) (in F a macrophage -M- beginning to penetrate through an interendothelial gap -arrow). G, H. Cytoplasmic processes of the pericytes incompletely cover the EC in a single layer (G) and overlap forming two or more layers (H). I. An activated pericyte showing a cilium (arrow) (higher magnification in the insert). Ultrathin sections. Uranyl acetate and lead citrate. A, x 12000; B, C, x 20000; D, x 15000; E, F, x 14000; G, H, x 13000; I, x 15000; I insert, x 20000
structures that cover gaps between endothelial cells (Figs. 1E,F). This is of interest for permeability regulation (see below).

Pericytes and lymphatic vessels

Lymphatic capillaries lack mural cells (Marchetti and Piacentini, 1990) and the basement membrane may be absent or discontinuous, whereas collecting lymphatic vessels show an SMC layer and a basement membrane. The factor Foxc2 and VEGFR3 signaling pathways probably interact in blocking mural cell recruitment in lymphatic vessels and thus they are required for the establishment of pericyte-free lymphatic capillary networks through the suppression of PDGF expression in EC (Petrova et al., 2004). Likewise, Foxc2 expression is induced by TGF-β (Petrova et al., 2004). Pericyte investment of lymphatic vessels occurs in Foxc2 deficiency (Foxc2−/− mice show abnormal lymphatic vascular patterning with increased pericyte investment of lymphatic vessels- Petrova et al., 2004).

Morphology

Pericyte morphology differs greatly depending on location (Joyce et al., 1984) and type of vessel. It may be irregular even in a single vessel. Frequently, pericytes show an elongated, stellate-shaped morphology (highly branched cells) with a cell body, nuclear region or perikaryon, from which arises an elaborate system of longitudinal and circumferential branches encircling the endothelium (Zimmerman, 1923; Weibel, 1974). The nuclear region or pericyte cell body is small and oval, and can be found immediately adjacent to the endothelium, and in a few cases protruding into the interstitial space. In this region, the pericytes show zonal rugged surface texture (Nakano et al., 2000). The major (primary or longitudinal) processes are orientated parallel to the long vascular axis and the smaller ones (secondary or circumferential) partially encircle the vessel wall. Besides, many small projections may extend from the margins of the primary and secondary processes (Nakano et al., 2000). Although the pericytic covering of endothelial cells is incomplete (Fig. 1G), the cytoplasmic processes of the pericytes sometimes overlap forming two or more layers (Fig. 1H) and in some conditions they appear dissociated from the EC, protruding away from the vessel or contributing cytoplasmic processes to more than one vessel (Weibel, 1974; Williamson et al., 1980; Mazanet and Franzini-Armstrong, 1982; Ando et al., 1999). A single pericyte in contact with several ECs suggests that they may integrate and mediate some EC functions (Gerhardt and Betsholtz, 2003; von Tell et al., 2006) and viceversa. According to location, pericytes change their shape, size, attachments, distribution and density. For example, compact mesangial cells in the kidney adopt a rounded morphology and show few attachments to the basement membrane and few contacts with abluminal vessel areas. Depending on vessel type, the pericytes have a highly elongated perikaryon with long, slender processes (capillaries) or a less extended nuclear region with thicker and more radial processes (post-capillary venules).

Structure

The pericyte cell body or nuclear region contains a prominent nucleus and reduced cytoplasm (Epling, 1966) (Figs 1A,E,G,H). Activated pericytes during angiogenesis shorten their processes and increase their somatic volume (see Behaviour of Pericytes During Angiogenesis).

Nucleus

The nucleus of pericytes is relatively large, in relation to the small content of cytoplasm in the cell body. Generally, the nucleus is kidney-shaped (Fig. 1A), protrudes towards the pericapillary space and shows dense patches of heterochromatin, above all in close proximity to the nuclear membrane (Fig. 1A,H). In activated pericytes, the nucleoli become prominent.

Cytoplasm

The perinuclear cytoplasm of pericytes is electron dense and in the vicinity of each pole of the nucleus usually contains small and slender mitochondria, few profiles of rough endoplasmic reticulum, a small population of free ribosomes, centrioles, non prominent Golgi sacules, multivesicular bodies, cytoplasmic vesicles and glycogen. The latter appear as dense cytoplasmic particles, arranged in clusters and less frequently aligned between smooth-surface cisternae of the endoplasmic reticulum (Bruns and Palade, 1968). Cilia have occasionally been described (Stensaas, 1975) (Fig. 1I), as well as osmiophilic bodies, which acquire lysosome characteristics in the central nervous system (Lafarga and Palacios, 1975; Sumner, 1982; Jeynes, 1985; Farrell et al., 1987). Depending on the number of lysosomes, the pericytes may be granular or agranular (filamentous) (Mato et al., 1980; Jeynes, 1985; Sims, 1986, 1991; Farrell et al., 1987; Tagami et al., 1991), (in the SNC, most are in fact granular -Farrell et al., 1987). The processes, with a circular or oval profile in cross section, measure from 0.05 to 0.4 μm in diameter (Bruns and Palade, 1968) and show some membranes of rough endoplasmic reticulum, free ribosomes, mitochondria and plasmalemmal vesicles, which are connected and continuous with the cell surface. Longitudinally oriented microtubules, 25 nm in diameter, intermediate filaments, 10 nm in diameter, and microfilaments, 7-8 nm in diameter, are also observed in these cells. The intermediate filaments, often in association with microtubules, are usually located within the cell bodies and in the abluminal portion of the proximal sites of the processes, but are absent in the most distal sites. The
Fig. 2. Pericyte surface and contacts. 

A. Numerous caveolae (arrows) located in the abluminal surface of a pericyte (P). EC: endothelial cell.

B. Type IV collagen around endothelial cells and pericytes (L: vessel lumen).

C-I. Pericyte/EC contacts (arrows) are shown including peg and socket arrangements, adherens junctions and gap junctions.

J. Anchoring junctions of a pericyte to the basement membrane (adhesion plaques) (arrowheads).

A, C-I: Ultrathin sections. Uranyl acetate and lead citrate. B: Immunohistochemical demonstration of collagen IV. A, C-I, x 22000; J, x 15000; B, x 250
individual or small groups of microfilaments extend from the cytoplasm of the pericyte body into the processes, even in the most distal sites. Frequently, the microfilaments originate a continuous plate of prominent bundles in the adluminal cytoplasm of the pericytes, coursing from one process to another, and forming a continuous “sole” applied against the curvature of EC (Bruns and Palade, 1968). Also present are dense bodies similar to those of smooth muscle cells and adhesion plaques or insertional dense plaques between microfilament bundles and dense material in the extracellular matrix (Rhodin, 1968; Vegge, 1972; Tilton et al., 1979a).

Surface

In normal resting pericytic microvasculature, pericytes show numerous caveolae or plasmalemmal vesicles, the majority located in the abluminal (adventitial) surface (Fig. 2A) (Frøkjaer-Jensen, 1984), principally in the perinuclear region and in the roots of the processes, wherein they appear usually single or forming few focal rows. The luminally orientated pericyte membrane and the tip of the processes are generally devoid of caveolae. The plasmalemmal vesicles have a diameter approximately of 700Å and show a membrane identical to the plasmalemma. The pericyte caveolae are believed not to be motile and their functions include discharge of plasma “cargo” into the intracellular space and incorporation of extracellular molecules to cell interior (potocytosis). Rare coated vesicles are also observed. Vesicle-associated membrane proteins (VAMPs), an important family of v-SNARES, have been located in pericyte caveolae of human venules, suggesting a role in vesicular containers that may exert their function while remaining attached to the plasma membrane. Caveolin-1 has been detected in pericytes (Virgintino et al., 2002).

Basement membrane

Pericyte cell bodies and cytoplasmic processes, as well as the ECs, are enveloped by the same basement membrane (Fig. 2B) (Cohen et al., 1980; Sims, 1986, 1991; Díaz-Flores et al., 1991a, 1992a; Schor et al., 1991; Mandarino et al., 1993), which they contribute to form (Mandarino et al., 1993). In the region where the basement membrane separates pericytes from endothelial cells, several fenestrae are observed, which permit direct contact between these cells (Rhodin, 1968; Tilton et al., 1979a; Sims, 1986). Although basement membrane is usually described as completely encircling the pericyte, except for where the tip of pericyte processes are closely apposed to the endothelium, it can be incomplete. Indeed, basement membrane may be absent in some regions between pericytes and adjacent ECs. Likewise, some cells, such as the denominated perivascular cells (Graeber et al., 1992; Kida et al., 1993) and granular perithelial cells (Ookawara et al., 1996) in the SNC, which have a pericytic morphology and position, are not completely surrounded by basement membrane and are considered a subpopulation of pericytes (Thomas, 1999).

The basement membrane, a network of fine filaments embedded in an homogenous and rich amorphous, electron-dense material (Kramer and Little, 1953; Yamada, 1955; Shakib and de Oliveira, 1966; Bruns and Palade, 1968), contains laminins (a large family of glycoproteins composed of α, β and γ chains), type IV collagen (Fig. 2B), nidogens (entactins) and heparin sulphate proteoglycans. In the microvascular basement membrane, two independent and distinct supramolecular three-dimensional networks are formed by laminin and type IV collagen, respectively (Yurchenko et al., 1992, 1993), both linked and stabilized by nidogens I and II, and the heparan sulphate proteoglycan perlecain (Timpl, 1996). Type IV collagen confers structural stability to the basement membrane and the different isoforms of laminins (isoforms laminin 8 -laminin α4β1 and γ1- and laminin 10 -laminin α5β1 and γ1- Hallmann et al., 2005) are biologically active with several signals in different tissues (Hallmann et al., 2005). Heparan sulphate proteoglycans cross-link the laminin and collagen type IV networks and bind soluble factors. Fibulins I and II bind two nidogens and laminins (Sasaki et al., 2002; Timpl et al., 2003). Other minor components include collagen types VIII, XV and XVIII, trombospondins I and II and osteonectin (Timpl, 1989; Hallmann et al., 2005).

The functions of basement membrane include (Hallman et al., 2005): a) structural support of EC and pericytes (vessel stability), b) separate tissue components, with selective filtration capacity, contributing a barrier to both soluble molecules and migrating cells, c) incorporation of soluble factors, such as VEGF and FGF-2 (Panayotou et al., 1989), d) substrate for cellular interactions (e.g interactions between EC and pericytes), with roles in cell migration, proliferation, differentiation and repair, and e) transduction of mechano-sensing signals from the vessel lumen to the vessel wall.

MMP-2 production by EC and pericytes acts on basement membrane and is associated with vessel sprouting (Virgintino et al., 2007).

Contacts and morphologic interrelations

Pericytes contact with ECs and with basement membrane, and they may establish morphologic interrelations with transmigrating cells. Pericyte processes do not frequently come into contact with each other, and specialized intercellular junctions between pericytes have not been noted.

Pericytes and ECs are in close proximity and they make frequent direct contact with each other (Fig. 2C-I) through interruptions in the basement membrane (pericytes are intimately adherent to the abluminal EC surface, unlike vascular SMC) (Sims, 1986; Carlson,
microvasculature (see below). Regulatory interactions of both the quiescent and angiogenic stages of the communicating and in regulation (positive and negative) paracrine signalling pathways, can participate in cell-cell intimate pericyte/endothelial contacts, in addition to the messengers (Fujimoto, 1995). Therefore, direct and metabolites and small molecules, including secondary connections between the cytoplasms of pericytes and closed or open hemichannel, providing direct through the intercellular space. Each connexon forms a arrays, composed of 6 connexin proteins. The connexons cluster into arrays, arranged in hexagonal lattice, in which the adjacent cell membranes appear to connect the cytoskeleton of pericytes and ECs, supporting transmission of mechanical contractile forces (Díaz-Flores et al., 1991a; Sims, 1986, 1991; Tilton, 1991; D’Amore, 1992; Shepro and Morel, 1993; Wakui et al., 1993; Fujimoto, 1995; Hirschi and D’Amore, 1996; Chakravarthy and Gardiner, 1999).

In peg and socket arrangements (Fig. 2C-E,F), cytoplasmic processes of pericytes and endothelial cells interdigitate after penetrating the basement membrane (Matsusaka, 1975; Leeson, 1979; Tilton et al., 1979a,b; Ho, 1985; Sims, 1986, 1991; Wakui et al., 1989; Díaz-Flores et al., 1991a; Shepro and Morel, 1993; Rucker et al., 2000). Generally, cytoplasmic fingers of the pericytes insert themselves into deep EC invaginations and may touch the endothelium establishing other types of junctions (Rucker et al., 2000). EC fingers inserting into pericyte invaginations are much less frequently observed.

The adherens junctions (Fig. 2D) are specialized areas that connect the cytoskeleton of pericytes and ECs through cytoplasmic membranes and intercellular material (e.g. mucopolysaccharides) by means of catenins and intracellular and extracellular transmembrane proteins (cadherins). They are similar to desmosoma, hold pericytes and EC, intervene in contact inhibition and provide strong mechanical attachment (anchor pericytes to ECs), supporting transmission of mechanical contractile forces (Díaz-Flores et al., 1991a; Sims, 1986, 1991; Hirschi and D’Amore, 1996).

The gap junctions (Fig. 2G-I) (Cuevas et al., 1984; Sims, 1986; Larson et al., 1987; Díaz-Flores et al., 1991a), in which the adjacent cell membranes appear to fuse or are separated by a 2 to 3 nm space, appear as clustered channels (connexion) arranged in hexagonal arrays, composed of 6 connexin proteins. The connexons of each cell membrane are lined up with one another through the intercellular space. Each connexon forms a closed or open hemichannel, providing direct connections between the cytoplasms of pericytes and EC, and allowing the passage of ionic currents, metabolites and small molecules, including secondary messengers (Fujimoto, 1995). Therefore, direct and intimate pericyte/endothelial contacts, in addition to the paracrine signalling pathways, can participate in cell-cell communication and in regulation (positive and negative) of both the quiescent and angiogenic stages of the microvasculature (see below). Regulatory interactions between Pericytes and EC). For example, cell-cell contacts are necessary for activation of some signals. Thus, gap junction communication, by means of connexons, mediates TGF-β activation and endothelial-induced mural-cell differentiation (Orlidge and D’Amore, 1987; Kruger et al., 2000; Hirschi et al., 2003).

Therefore, in these junction complexes, several components are present, such as extracellular matrix, cell adhesion molecules, N-cadherin (Heimark, 1993; Gerhardt et al., 2000; Gerhardt and Betsholtz, 2003), β catenin-based adherent junctions and fibronectin. N-cadherin expression in pericytes and EC mediates pericytic-endothelial interactions, represents an initial signal involved in blood-brain barrier development and increases in angiogenic vessels (Gerhardt et al., 1999, 2000). Fibronectin is present in areas of pericyte-endothelial cell apposition, where fine cytoplasmic fibrils appear to insert themselves into the pericyte plasma membrane (Courtoy and Boyles, 1983). This fact suggests a mechanical linkage between apposed cell surfaces. Indeed, pericyte/endothelial contacts make up a structural mechanism for contractile force transmission and a possible receptor system in which the pericytes and endothelial cells respond to secondary signals generated by themselves or by other cells (Davies, 1986). For example, ECs produce endothelin 1, which stimulates pericyte contraction (Chakravarthy et al., 1992; Rucker et al., 2000).

Selective disruption of pericyte/endothelial cell contacts is a characteristic finding of diabetic retinopathy (Cogan et al., 1961; Kuwabara and Cogan, 1963; De Oliveira, 1966; Speiser et al., 1968; Ashton and Tripathi, 1977; Sima et al., 1985; Robinson et al., 1989). This feature could lead to loss of capillary tone, resulting in dilatation, formation of microaneurysms and angiogenic activity (Cogan and Kuwabara, 1967; Robinson et al., 1989) (see below).

Anchoring junctions of pericytes to the basal membrane (adhesion plaques) (Fig. 2J) are specialized areas where bundles of cytoplasmic microfilaments terminate in the plasma membrane and attach to integrins (transmembrane linkers). The latter, through their extracellular domains, attach to extracellular matrix proteins.

Pericytes establish morphologic interactions with transmigrating leukocytes, mainly monocytes (macrophages). We have observed that, during angiogenesis and granulation tissue formation, macrophages contribute to the dissociation and detachment of pericytes from the parent vessel wall, like a tug (macrophage) towing a ship (pericyte-myofibroblast). This topic requires further research (see below).

Although SMCs, in contrast to pericytes, do not appear to communicate with EC by direct physical contacts, during vascular sprouting from rat femoral vein induced “in vivo” by glycerol and prostaglandins E1 and E2, we have demonstrated that SMCs in the media acquire a pericyte phenotype (Fig. 3A,B). Indeed, these
cells extend cytoplasmic processes, which cross through the gaps of the discontinuous internal elastic lamina, penetrate the intima and fit into EC foldings (Fig. 3B), resembling peg and socket contacts between pericytes and EC (Díaz-Flores et al., 1994, 1996).

**Identification, macromolecules expressed by pericytes and pericyte markers**

The cellular population in a perivascular location of the microvasculature is heterogeneous (Sims, 2000). Therefore, its identification remains difficult because it represents a continuum of mural phenotypes or separate cell types with different functions in various tissues. Moreover, several types of pericytes may co-exist in the same vascular bed (Kurz et al., 2008). In addition to morphologic and structural criteria (embedded into a basement membrane, in close association with ECs, and presence of contacts with ECs), the expression of several markers is important for the identification of pericytes, although none of them is specific or a general panpericyte marker (none recognizes all pericytes) (Armulik et al., 2005; Edelman et al., 2006). Furthermore, the markers are not continually expressed in the pericytes or in their precursor and descendant cells. Thus, the expression of markers depends, among other factors, on the following: a) Species (e.g. expression of alpha SMA in pericytes derived from the chicken embryo brain and absence of expression in those derived from the mouse embryo brain and human brain - Gerhardt et al., 2000), b) Type of vessel or segment of the microvasculature (e.g. different expression of alpha SMA - Nehls and Drenckhahn, 1993) (see below), c) Quiescent or angiogenic stages of the blood vessels, d) Local anatomy, with marker pattern variation in different organs (in a tissue-specific manner), and e) Pathological conditions (Sundberg et al., 1993; Buschard et al., 1996; Schlingemann et al., 1996; Dvorak and Feng, 2001). This phenotype instability increases “in vitro” with both morphology and molecular expression diversity.

Pericytes or subsets of pericytes may express a great number and variety of macromolecules, including Growth Factors, Receptors, Cytokines, Enzymes and Adhesion Molecules (Table 1).

The principal pericyte markers are antibodies against cell-surface proteins and intracellular proteins (Joyce et al., 1985a; Schlingemann et al., 1990; Nehls and Drenckhahn, 1991; Nehls and Drenckhahn, 1993; Sundberg et al., 1993; Buschard et al., 1996; Alliot et al., 1999; Hellstrom et al., 1999; Bergers and Song, 2005). Some of these macromolecules, such as growth factors and growth factor receptors, will be considered in the section of pericyte interactions. Here, we will only take into account some markers as follows: A) Contractile proteins: α-SM actin (Fig. 3C,D) (a cytoskeletal protein present in cells of smooth muscle lineages and myofibroblasts), desmin (Fig. 3E) (a muscle specific class III intermediate filament), tropomyosin and other contractile proteins (Joyce et al., 1985a,b; Herman and D’Amore, 1985; DeNofrio et al., 1989; Skalli et al., 1989; Darby et al., 1990; Nehls and Drenkhnahn, 1991; Nehls et al., 1992; Schurch et al., 1997; Orte et al., 1999). α-SM actin is located in microfilament bundles of the pericytes (Skalli et al., 1989) of the pre and postcapillary vessels (Smooth muscle transitional type – (Nehls and Drenckhahn, 1991) of the retina and mesentery), whereas it is absent in mid-capillaries (Nehls and Drenckhahn, 1993). Therefore, according to α-SM actin expression, the pericytes may have a capillary phenotype (α-SM actin negative and desmin positive) or a venular phenotype (both α-SM actin and desmin positive). In this way, capillary pericytes in culture (Nehls and Drenckhahn, 1993) and in presence of TGF-β (Verbeek et al., 1994) begin to express α-SM actin. B) Regulator of G protein signalling 5 (RGS-5) (Bondjers et al., 2003; Cho et al., 2003; Berger et al., 2005), a GTPase activating protein (Anger et al., 2004).

It is the earliest gene activated in pericytes during angiogenesis (Bondjers et al., 2003). RGS also controls the differentiation of chondroblasts (Appleton et al., 2000), osteoblasts (Thirunavikkarasu et al., 2002) and SMCs (Cho et al., 2003; Li et al., 2004). C) Some intermediate filaments such as nestin (Alliot et al., 1999). D) NG2, neuron-glial 2, high-molecular weight melanoma-associated antigen or HMWMAA, is a chondroitin sulphate proteoglycan expressed in the plasma membrane surface of arteriolar and capillary pericytes (absent in venular pericytes) (Schlingemann et al., 1990; Murfee et al., 2005) and during vasculogenic and angiogenic processes (Ozerdem et al., 2001; Stallcup, 2002; Ozerdem and Stallcup, 2003). E) MxS1, one of the genes that encode homeodomain transcription factors, which are essential for vascular mural cell formation and remodelling. It is highly expressed in arterioles and capillaries and is a novel marker for a subpopulation of pericytes (Goupille et al., 2008). F) Anexin 5-LacZ fusion gene (Anxa5-LacZ), which is expressed in perivascular cells in embryonic and adult tissues. G) Endosialin, a C-type, a lectin like cell surface receptor (Christian et al., 2001). H) VCAM-1 (Vascular cell adhesion molecules-1) or CD-106, expressed in ECs and pericytes, acts in cell adhesion and chemotaxis signal transduction (Carter and Wicks, 2001). I) 3G5 is a pericyte-associated cell surface antigen, which is also highly expressed by a large proportion of haematopoietic marrow cells (Shi and Grontos, 2003). J) 130 KDa protein and aminopeptidase N, a 140 KDa protein, recognized by monoclonal antibodies in the pericyte plasma membrane surface (Krause et al., 1988; Kunz et al., 1994). In this way, pericytes and periendothelial cells in all brain parenchyma vessels of all sizes have shown immunoreactivity for aminopeptidase N and aminopeptidase A (Alliot et al., 1999).

**Pericyte functions**

Several functions of pericytes in physiological and pathological repair processes include: a) vessel stabilization (supplying structural integrity of microvessels and mediating EC quiescence -von Tell et
Fig. 3. SMC acquiring pericyte phenotype, and cytoskeletal markers in pericytes. A, B. Angiogenesis from femoral vein. A. Vascular sprouts (arrows), one of them crossing the internal elastic lamina (arrowheads). B. Smooth muscle cells (SMC) extending cytoplasmic process through the gaps of elastic lamina (EL), penetrate the intima and fit into endothelial cell (EC) foldings (arrows), resembling peg and socket contacts (like pericytes) (L: vessel lumen). C-E. Actin (C and D) and desmin (E) expression in pericytes. F. Vimentin is present in pericytes and ECs. A: Semithin section, Toluidine Blue. B: Ultrathin section, Uranyl acetate and lead citrate. C-F: Immunohistochemical demonstration of actin, desmin and vimentin, respectively. A, x 350; B, x 13000; C-F, x 250
Table 1. Molecules expressed by pericytes.

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<tr>
<th>Growth factors</th>
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<tr>
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<tr>
<td>Ang-1</td>
<td>Angiopoietin-1</td>
</tr>
<tr>
<td>CCL2</td>
<td>Glioma-derived monocyte chemotactic F-2</td>
</tr>
<tr>
<td>CD248</td>
<td>Endosialin</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Stromal cell-derived F</td>
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<tr>
<td>gas-6</td>
<td>Aoxi stimulatory F</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony stimulating F</td>
</tr>
<tr>
<td>HB-EGF</td>
<td>Heparin binding E</td>
</tr>
<tr>
<td>IGF-2</td>
<td>Insulin-like F-2</td>
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<td>IGFBP2,3,7</td>
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</tr>
<tr>
<td>ILF</td>
<td>Leukemia inhibitory F</td>
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<tr>
<td>osteoglycin</td>
<td>Osteoinductive F</td>
</tr>
<tr>
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<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
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</tr>
<tr>
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<td>Placenta GF</td>
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<tr>
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<td>Regulator of G-protein signaling 5</td>
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<tr>
<td>TF</td>
<td>Tissue F</td>
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<tr>
<td>VEGF-B, C</td>
<td>Vascular endothelial GF B, C and D</td>
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<td>TMSB10</td>
<td>Thromosin [β10]</td>
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<tr>
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<tr>
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<td>Insulin-like GF-1</td>
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<tr>
<td>M-CSF</td>
<td>Macrophage colony stimulating F</td>
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<tr>
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<td>Nerve GF</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived GF</td>
</tr>
<tr>
<td>PEDF</td>
<td>Pigment epithelium-derived F</td>
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<tr>
<td>TGF-β</td>
<td>Transforming GF-β</td>
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<td>VEGF</td>
<td>Vascular endothelial GF</td>
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<td>Small family of zinc-finger transcription factors</td>
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<th>Other macromolecules</th>
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<td>IL1 (α &amp; β)</td>
<td>Interleukin-1 (α &amp; β)</td>
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<tr>
<td>IL6</td>
<td>Interleukin-6</td>
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<tr>
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<td>Adrenomedullin</td>
</tr>
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<td>CD249</td>
<td>Aminopeptidase A</td>
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<tr>
<td>CD73</td>
<td>Lymphocyte-vascular adhesion protein-2</td>
</tr>
<tr>
<td>CD13</td>
<td>Membrane aminopeptidase</td>
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<tr>
<td>Klotho</td>
<td></td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitor metalloproteinases</td>
</tr>
<tr>
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<td>Tenascin C</td>
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<tr>
<td>Thrombospondin 1</td>
<td>Thrombospondin 1</td>
</tr>
<tr>
<td>TIMP 1 &amp; 4</td>
<td>Tissue inhibitor metalloproteinases 1 &amp; 4</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone morphogenetic protein</td>
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<td>collagen IV</td>
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<td>collagen 18</td>
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<tr>
<td>FMD</td>
<td>Fibromodulin</td>
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<td>Integrin-alpha-5</td>
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<tr>
<td>CD166</td>
<td>Activated leukocyte cell AM</td>
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<td>ITGA5</td>
<td>Integrin-alpha-5</td>
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Pericytes and mesenchymal cell niche
al., 2006), b) regulation of vascular tone and maintenance of local and tissue homeostasis (contractile capacity, transport and permeability regulation), c) synthesis of matrix proteins, d) macrophage-like properties, e) activity in immunologic defense, f) intervention in coagulation, g) participation in regulatory mechanisms that govern quiescent and angiogenic stages of blood vessels (including those related to the behaviour of pericytes during angiogenesis, and pericyte interactions with other cells and with the extracellular matrix) and h) mesenchymal potentiality (including pericyte function as progenitor cells and participation in a perivascular mesenchymal stem cell niche).

**Vessel stabilization**

Vascular stabilization or maturation is defined as the maintenance of established vasculature and the envelopment of pericytes or smooth muscle cells around the EC layer during angiogenesis (Hirschi and D’Amore, 1997; Jain, 2003; Paik et al., 2004). Recruitment of pericyte and formation of extracellular matrix, including the basement membrane, are important for blood vessel stabilization and function (Allt and Lawrenson, 2001; Armulik et al., 2005). In this way, pericyte processes may conform small vessel shapes and contour, and, along with the layers of basement membrane (which they contribute to form), may provide mechanical support for the capillary wall, preventing excessive dilatation, with direct correlation between numbers of pericytes and resistance of the microvasculature to high blood pressure (Latives et al., 1979). Therefore, pericytes are crucial for vascular diameter regulation and functional patterning (Benjamin et al., 1998; Hellström et al., 2001; Gerhardt and Betsholtz, 2003). Besides, pericyte/EC contacts promote EC survival through secretion of VEGF and Ang1 (Benjamin et al., 1998; Reimnuch et al., 2001) and regulate EC migration, proliferation, differentiation and branching (Carmeliet, 2003a; Hirschi et al., 1998) (see below).

**Regulation of vascular tone and maintenance of local and tissue homeostasis**

Pericytes maintain local and tissue homeostasis by vessel caliber modification and by transport and capillary permeability regulation.

**Contractile capacity with vasoconstriction and vasodilatation (vessel caliber modification)**

Pericytes are considered contractile cells (Rouget, 1873; Zimmermann, 1923; Bensley and Vimtrup, 1928; Tilton et al., 1979b; Kelley et al., 1987; Hirschi and D’Amore, 1996; Rucker et al., 2000; Gerhardt and Betsholtz, 2003; Peppiatt et al., 2006; Yamanishi et al., 2006; Kutcher et al., 2007) and their microvascular vasomotion acts in hemodynamic regulation of microvascular blood flow and permeability, either increasing or reducing endothelial cell junctional inflammatory leakage.

The pericyte capacity to contract or relax is supported by the following (Díaz-Flores et al., 1991; Nehls and Drenckhahn, 1993; Shepro and Morel, 1993; Sims, 2000): a) characteristic location and morphology, with multiple primary processes that envelop and compress the EC, as well as with the presence of a rugged surface texture of the pericyte cell body showing lamellar folds in scanning electron microscopy (Nakano et al., 2000), b) ultrastructural demonstration of distinctive cytoskeletal elements (an elaborate contractile apparatus), c) immunohistochemical expression of contractile proteins (Herman and D’Amore, 1985; Joyce et al., 1985b; Fujimoto and Singer, 1987), such as actin (Wallow and Burnside, 1980; Kelley, et al., 1987; Das et al., 1988; Graeber et al., 1989) (although with a different expression – see above), myosin (Joyce et al., 1985a,b), tropomyosin (Joyce et al., 1985a), as well as of cyclic GMP-dependent protein-kinase (involved in contraction regulation) (Movat and Fernando, 1964; Joyce et al., 1984, 1985a,b); the different expression in pericytes of contractile proteins is probably associated with the blood flow requirements in the tissues (Tilton et al., 1979b), d) presence of receptors for several molecules that regulate contractility, such as endothelin receptors (the vasoconstrictor endothelin is produced by the endothelial cells (Takahashi et al., 1989; Masaki, 1995), cholinergic and adrenergic alpha 2 and B2 receptors (Rucker et al., 2000) and for other vasoactive substances, such as angiotensin II (Bergers and Song, 2005, Speyer et al., 1999) and e) demonstration of excitability with production of action potentials (Helbig et al., 1992), in response to vasoactive agents (Tilton et al., 1979b; Lee et al., 1989; Chakravarthy et al., 1991; Helbig et al., 1992; Ferrari-Dileo et al., 1996), and reduction in diameter and selective buckling of endothelial cells beneath the pericytes. For example, contraction in rat cardiac and skeletal muscle microvasculature (Tilton et al., 1979b; Joyce et al., 1985b) and in “in vitro” experiments (Kelley et al., 1987). The contraction in response to vasoactive agents has been measured by several authors (Tilton et al., 1979a; Kelley et al., 1987; Hirschi et al., 1998), and the vasoconstriction or vasodilatation depends on the agent and on the duration of the exposure (Edelman et al., 2006a). Thus, endothelin, norepinephrine, angiotensin II, serotonin and bradikinin produce strong contraction of pericytes, while adrenergic agonists (B2), nitric oxide, atrial natriuretic peptide, adenosine, lipopolysaccharide and reactive oxygen metabolites induce relaxation (Lee et al., 1989; Chakravarthy et al., 1991; Helbig et al., 1992; Haefliger et al., 1994; Anderson and Davis, 1996; Ferrari-Dileo et al., 1996; Zschauer et al., 1996; Chen and Anderson, 1997; Haefliger and Anderson, 1997; Matsugi et al., 1997; Edelman et al., 2000a; Speyer et al., 2000; Speyer et al., 2002; Edelman et al., 2006b). For example, primary culture brain pericytes are relaxed by
adrenomedullin, with increased intracellular cAMP concentration and decreased phosphorilation of myosin light chain (Takata et al., 2008).

Unlike smooth muscle cells, pericytes constrict the endothelium by localized buckling (localized deformation of EC wall), as they do not completely encircle the ECs (Nakano et al., 2000).

Besides, pericytes release factors that produce constriction by controlling expression of nitric oxide synthase and endothelin-1 in microvascular ECs (Martin et al., 2000).

Several pericyte contractile functions include (Shepro and Morel, 1993) regulation of blood flow and perfusion (Forbes et al., 1977; Tilton et al., 1979a; Ushiwata and Ushiki, 1990; Tilton, 1991; Shimada et al., 1992), aperture of interendothelial junctions (Braverman and Braverman, 1986; Murphy and Wagner, 1994) and junction protection from overstretching ("umbrella-like" disposition over interendothelial junctions- Sims, 1991).

Transport and capillary permeability regulation
Pericytes participate as regulators of capillary and venular permeability by changing the amount of EC abluminal surface covered (Murphy and Wagner, 1994) or by EC intercellular junction action (Cuevas et al., 1984; Miller and Sims, 1986; Shepro and Morel, 1993), whose open state may be affected by the capacity of pericyte contraction (Sims and Westfall, 1983; Buchanan and Wagner, 1990; Murphy and Wagner, 1994; Kim et al., 1998; Allt and Lawrenson, 2001) (see above). Furthermore, the presence of pericytes around endothelial cell junctions, adopting an umbrella-like disposition (Fig. 1E,F), holds proteins and cells within the vessel wall (control of fluid transfer and cell movement) (Fig. 4A,C) (Sims and Westfall, 1983; Sims et al., 1990, Sims et al., 1994; Rodriguez-Baeza et al., 1998; Sims, 2000). An excellent method to demonstrate both this control of fluid transfer and the capacity of retention of certain substances (e.g. Monastral Blue) by pericytes and vessel basement membrane is based on the intravenous injection of the substance followed by local administration of histamine (Fig. 4A,B). In this way, projections derived from the secondary processes depress the ECs in areas corresponding to the interendothelial junctions (Nakano et al., 2000). Likewise, pericytes secrete prostaglandins (e.g. prostacyclin), which induce vasodilatation (Eskenasy and Tasca, 1988; Hudes et al., 1988). Central nervous system microvascular pericytes contribute to the formation of the blood-brain barrier (Balabanov and Dore-Duffy, 1998) with intervention of transforming growth factor β (Dohgu et al., 2005) and angiopoietin (Hori et al., 2004). Moreover, pericytes present mRNA for VEGF (Arello et al., 1995) and they may also increase permeability of the endothelial barrier through increased VEGF production (Sprague et al., 1990; Stephenson et al., 1990; Wurtz et al., 1992; Lonigro et al., 1996; Kim et al., 1998).

Therefore, the presence of supporting pericytes during the quiescent stage of microvasculature prevents leakage through the vessel wall, reducing the permeability of the ECs, whereas defective pericyte coverage during angiogenesis (repair, tumors or other human diseases) induces a poorly organized and leaky vasculature. For example, pericytes are crucial in the development of capillary leak and pulmonary edema in acute respiratory distress syndrome (Edelman et al., 2006a,b).

**Synthesis of matrix proteins**
Pericytes contribute to the formation of extracellular matrix components, including the vessel basement membrane. Furthermore, they synthesize numerous molecules (see above).

**Macrophage-like properties**
Macrophage-like potentiality of some subsets of pericytes or pericyte-like perivascular cells has been described and become overtly so in inflammatory reactions (Cotran and Majno, 1964; Majno and Palade, 1961; Thomas, 1999). These properties include the following: a) uptake capacity of soluble and small molecules (pinocytosis) (Kristensson and Olsson, 1973; van Deurs, 1976; Mato et al., 1980, 1982, 1985; Mato and Ookawara, 1981), b) phagocytosis (Majno et al., 1961; Kristensson and Olsson, 1973; Van Deurs, 1976; Castejon, 1984; Jeynes, 1985; Kida et al., 1993; Mato et al., 1994; Balabanov et al., 1996; Bergers and Song, 2005), with scavenger receptors and capacity to incorporate macromolecules “in vivo” (Fig. 4D,F) and in culture (Balabanov et al., 1996, Mato et al., 1996; Honda et al., 1998; Bergers and Song, 2005), c) possible conversion into macrophages (Maxwell and Kruger, 1965; Baron and Gallego, 1972; Boya, 1976) and d) expression of markers of macrophages, including CD4, leukocyte-common antigen, complement receptor, Fc receptor, L368, class I and II major histocompatibility complex molecules, CR3 complement receptor and ED-2 (Balabanov et al., 1996; Thomas, 1999). The pericytes as a pool of macrophage-like cells have been widely studied in brain vasculature (Balabanov et al., 1996; Hirschi and D’Amore, 1996; Thomas, 1999) (see immunologic defense), and conversion of pericytes into macrophages, including microglia, have also been pointed out (Maxwell and Kruger, 1965; Baron and Gallego, 1972; Matthews and Kruger, 1973; Boya, 1976, Brierley and Brown, 1982, Jordan and Thomas, 1988, Monteiro et al., 1996).

**Immunologic defense**
Pericytes can act as antigen-presenting cells for primed T-lymphocytes via the expression of MHC class II (Fabry et al., 1993a; Balabanov et al., 1999; Guillemin and Brew, 2004) and are the first line of immunologic defense in the brain. The fraction of pericyte-like cells that behave as macrophages (pericytal macrophages),
Fig. 4. Morphologic expression of some pericyte functions. **A-C.** Pericyte control of fluid transfer and cell movement. **A, B.** Intravenous injected Monastral Blue retained (arrows) between pericytes (P) and endothelial cells (EC) after vascular leakage induction by local administration of histamine. **C.** A pericyte holds a macrophage (M) within the vessel wall. **L.** vessel lumen. **D-F.** Demonstration of macrophagic properties of pericytes. Exogenous dental material (arrows) is present in pericytes of vessels near to a treated tooth (in F, pericytes with the exogenous material show actin expression). **A** and **C.** Semithin section. Toluidine Blue. **B:** Ultrathin section. Uranyl acetate and lead citrate. **D and E:** H&E. **F:** Actin expression. **A, D-F, x 250; B, x 12000; C, x 450.**
contributing along with lymphocytes to an immune response (Thomas, 1999), may originate from the bone marrow mesenchymal progenitors, named fibrocytes (Díaz-Flores et al., 2009). Indeed, BM-derived precursor cells have been shown to give rise to a subset of pericytes (Rajantie et al., 2004; Ziegelhoeffer et al., 2004; Ozerdem et al., 2005) and between these precursor cells, the fibrocytes, expressing markers of leukocytes (CD-45, LSP1) and monocyte lineage (CD-11a, CD-11b, CD-13, CD-32, CD-64), have the ability to present antigen “in vitro” and “in vivo”.

Intervention in coagulation

The strategic position of pericytes facilitates their participation in coagulation after microvascular injury or rupture (Thomas, 1999). In this way, they regulate the expression of procoagulant enzyme complexes, comprising the extrinsic pathway of blood coagulation (Bouchard et al., 1997) through tissue factor (the primary cellular initiator of the extrinsic coagulation protease cascade) of which pericytes are a source (Bach and Rifkin, 1990).

Pericyte function in regulatory mechanisms that govern the quiescent and angiogenic stages of blood vessels

Pericytes have an important role in the mechanisms that govern the quiescent and angiogenic vascular stages. Understanding these mechanisms is crucial to understand pericyte behaviour in these stages. Previously, we have described pericytes in the quiescent microvasculature, as well as their origin during vasculogenesis in embryonic development. Below we consider pericyte interactions with other cells and with extracellular matrix, after describing the behaviour of pericytes during sprouting angiogenesis and intussusceptive angiogenesis.

Behavior of pericytes during sprouting angiogenesis

Angiogenesis, neovascularization or formation of new blood vessels from the established microcirculation, is a complex process and includes the following findings: a) EC and pericyte activation, b) proteolytic degradation of the basement membrane (remodelling at a molecular level, rather than complete dissolution), c) EC migration and proliferation, d) detachment, migration and proliferation of pericytes, e) formation of a new capillary vessel lumen, f) recruitment of pericytes around the new capillaries, g) development of a new basement membrane and h) vascular maturation and remodelling, with involution of most newly formed vessels. An inflammatory response may precede and accompany the process. Furthermore, during angiogenesis and vascular involution, changes in the interstitium may occur, such as contribution of matrix-forming cells (fibroblast/myofibroblasts, osteoblasts, chondroblasts), contractile cells (SMC, myofibroblasts and myointimal cells) and adipose cells. Next, we will consider pericyte behaviour in pre-existing vessels (pericyte/EC dissociation), in the early angiogenic sprouts, in involutive vessels and in stabilizing vessels.

Pericytes in pre-existing vessels. Vessel destabilisation.

Pericyte/EC dissociation. In response to proangiogenic factors, such as VEGF (see below), capillaries and postcapillary venules show vasodilatation and increased vascular permeability. During the initial phase of angiogenesis (Fig. 5A) (Díaz-Flores et al., 1992a, 1994), activated pericytes in pre-existing vasculature (parent vessels) bulge, shorten their processes and increase their somatic volume (Fig. 5B-E), adopting an angiogenic phenotype. Their nuclei display some folding and the nucleoli become prominent (Fig. 5B). The cytoplasm acquires numerous ribosomes, either singly or in aggregates, and multiple profiles of rough endoplasmic reticulum are observed. Micropinocytic vesicles are widely distributed along the cell membrane. Likewise, a sudden and intense pericyte proliferation occurs (Fig. 5C and insert). In autoradiographic studies, numerous 3H labelled pericytes are observed (Fig. 5C insert) (Schoeffl, 1963; Cavallo et al., 1972, 1973; Sholley et al., 1977; Burger and Klintworth, 1981; Díaz-Flores et al., 1992a, 1994). Frequently, the pericyte basement membrane is disrupted and fragmented, sometimes away from the pericyte cell surface. Many pericytes project into the perivasculare space (Fig. 5E) and appear detached from the vessel walls. Pericyte dissociation may be facilitated by migrating macrophages (Figs. 4C, 5D). In this stage, the number of pericytes in the parent vessels decreases, pericyte/endothelium surface contacts become markedly lower, and the mean separation between the pericyte and ECs is greater (Díaz-Flores et al., 1992).

Cell forms transitional between activated pericytes and fibroblast/myofibroblasts (Fig. 5F) that may or may not have a partial basement membrane and are also observed. The nucleus of these fibroblast-like cells is large, principally ovoid, rich in euchromatin and has one or two prominent nucleoli. The cytoplasm contains abundant RER, ribosomes, a Golgi complex and slender mitochondria. Some of these cells contain bundles of microfilaments (40-80 Å), with focal densities (dense bodies) similar to those of myofibroblasts. In summary, the pericytes of the pre-existing vessels become plump, ameboid and mitotically active (Díaz-Flores et al., 1991a; Movat and Fernando, 1964; Cotran, 1967). The hypoxic state stimulates pericyte migration and angiogenesis (Murata et al., 1994). After specifically labelling cells in the pericytic microvasculature walls, during angiogenesis, the marker continues to be observed in pericytes and endothelial cells of the newly formed vessels, transitional cells between pericytes and fibroblasts and typical fibroblasts in the interstitium (Díaz-Flores et al., 1992a). These findings have provided greater evidence that preformed vasculature pericytes are substantially activated during postnatal angiogenesis and granulation tissue formation, and that they may
Fig. 5: Pericytes in pre-existing vessels during angiogenesis. A. Regions of pre-existing vessels (PV) and sprouting vessels (neovascularization: NV) are easily distinguishable during neovascularization from a vascular tissue to an avascular structure: cornea. B-F. Pericyte dissociation from the vessel wall. Pericytes (P) shorten their processes, increase their somatic volume and project into the perivascular space (B-E) with basement membrane disruption (a pericyte mitosis is observed in C and, in the insert, ^3^H labelled pericytes are shown -arrow). EC: Endothelial cell. L: vessel lumen. Pericyte dissociation is frequently facilitated by migrating macrophages (D-M). F. A dissociated and detached cell shows a transitional phenotype between pericytes and myofibroblasts (TC). A, B and insert of C: Semithin sections. Toluidine blue. C to F: Ultrathin sections. Uranyl acetate and lead citrate. A, x 150; B, x 400; C-F, x 15000; insert of C, x 250
Pericytes contribute to the origin of new pericytes and fibroblasts (Díaz-Flores et al., 1992a).

When the pre-existing vessels return progressively to quiescent morphology, their pericytes, although still enlarged, show decreased ³H labelling indices and scarce mitotic figures.

**Pericytes in the early angiogenic sprouts.** Pericytes participate in early angiogenesis (Rhodin and Fujita, 1989; Schlingemann et al., 1990, 1991; Díaz-Flores et al., 1991a, 1992a, 1994; Schlingemann et al., 1991; Nehls et al., 1992; Wesseling et al., 1995; Schlingemann et al., 1996; Morikawa et al., 2002; Gerhardt and Betsholtz, 2003; McDonald and Choyke, 2003), associating with EC sprouts (Fig. 6). This recruitment and investment includes migration, alignment, contacts and phenotype changes. Although initially EC sprouts may form without pericycle involvement (representing a plasticity window allowing ECs to remodel), pericytes are also among the first cells to invade newly vascularized tissues (Díaz-Flores et al., 1991; Nehls et al., 1992; Reynolds et al., 2000) and some of them locate at the growing front of the endothelial sprouts (Fig. 6B and insert), participating in angiogenesis by determining the location of sprout formation and by guiding newly formed vessels (Nehls et al., 1992; Tazuki and Sasa, 1994; Ozerdem et al., 2001; Morikawa et al., 2002; Ozerdem and Stallcup, 2003). Therefore, there are two possibilities: 1) endothelial tube formation is followed by investment of pericytes, which use EC sprouts as migration clues. At the same time, there is expression of Ang-1/PDGF (see below) (Nicosia and Villaschi, 1995; Hashizume and Ushiki, 2002; Hoffman et al., 2005). 2) pericytes are the first to extend beyond the sprouting EC, promoting EC survival and guiding EC migration (Amselgruber et al., 1999; Morikawa et al., 2002; Darland et al., 2003; Kale et al., 2005). Besides, pericytes and macrophages can invade tissues in the absence of ECs and can form tubes (Moldovan et al., 2000; Anghelina et al., 2002, 2004, 2006; Ozerdem and Stallcup, 2003), enabling the posterior penetration of ECs. In this case, pericytes and macrophages act as a scaffold along which ECs migrate during sprouting.

**Pericytes in involutive vessels (in vessel regression) during angiogenesis.** In some forms of angiogenesis a dense network of neovessels is created in excess, and many newly-formed vessels undergo involution (Fig. 7). This massive regression of capillaries originates capillary-free zones and marked reduction in overall vascular density (Benjamin et al., 1998). Although pericycle coating may prevent vessel regression (Benjamin et al., 1998), we have observed pericytes in immature, mature and involutive microvessels (Fig. 7). Indeed, in most involutive vessels, the ECs disintegrate drastically as a result of apoptosis, whereas cells with pericycle characteristics persist (Fig. 7C-E). At first, numerous disintegrating vessels show marked intravascular accumulation of platelets (platelet thrombus) (Fig. 7A-D). We have hypothesized that this accumulation of factor-releasing platelets during the massive regression of neocapillaries converts the granulation tissue into a “paracrine transitional organ”, facilitating fibroblast/myofibroblast proliferation (Díaz-Flores et al., 2009). Subsequently, homogenized platelets, endothelial cell debris and basal membrane residues are observed (Fig. 7D,E).

**Pericytes in stabilizing vessels.** The non-regressing immature newly-formed vessels must mature at the levels of the vessel wall and of the network that they form. Maturation of the persistent vessel wall involves recruitment of pericytes with mesenchymal cell differentiation, surrounding extracellular matrix formation and organ-specific specialization.

Pericytes are recruited in the vascular sprouts in response to growth factors, such as PDGF (see below), and the new vessels stabilize (Balabanov et al., 1996). This association is a key to vascular maturation during remodelling and for vascular patterning and diameter regulation (Hellström et al., 2001; Gerhardt and Betsholtz, 2003; Betsholtz et al., 2005; Gerhardt and Semb, 2008). Although the mere presence of pericytes appears not to protect vessels from undergoing aggression after inhibition of VGFG (Morikawa et al., 2002; Inai et al., 2004), pericyte coverage intervenes favourably in vessel protection during development (Chan-Ling et al., 2004) and prevents vessel regression (Benjamin et al., 1998, 1999; Enge et al., 2002). Pericyte-endothelial maturation appears to be more important than mere pericyte coverage (Hoffmann et al., 2005).

**Behaviour of pericytes during “intussusceptive” microvascular growth.**

Pericytes participate in intussusceptive or splitting angiogenesis (non-sprouting angiogenesis) by means of transluminal pillars and folds (Patan et al., 1993, 1996; Djonov et al., 2000a,b, 2002, 2003; Burri et al., 2004; Kurz et al., 2008). Indeed, after two opposing endothelial walls of one capillary contact to form a fold and the junctions are reorganized, pericytes perforate the vessel bilayer, secrete extracellular matrix and generate a core that is fleshed out. One pericyte may contribute to the formation of more than one pillar. Likewise, pericytes actively intervene in pillar maturation via their numerous cell processes (Kurz et al., 2005).

**Pericyte interaction with endothelium and other cells, and with extracellular matrix.**

Pericytes, along with endothelium and other cells (tissue and transmigrating cells), as well as with plasma and extracellular matrix components, participate in regulation of quiescent and angiogenic stages of blood vessels by means of several molecules and pathways, including vasoactive and growth factors, adhesion
Fig. 6. Pericytes in early angiogenic sprouts. A-C and inserts. Incorporation of activated pericytes (P) and transitional cell forms between pericytes and myofibroblasts (TC) in early angiogenic sprouts (AS). PV: Pre-existing vessels. EC: endothelial cell. In B and insert, pericytes are located at the growing front of the endothelial sprouts. Inserts: Semithin sections. Toluidine blue. A-C: Ultrathin sections. Uranyl acetate and lead citrate. A-C, x 15000; inserts, x 250

Fig. 7. Pericytes in involutive vessels during angiogenesis. A-C. Involutive vessels with platelet thrombus (T) (converting granulation tissue into a "paracrine transitional organ"). P: Pericyte. EC: endothelial cell. D. Degenerative phenomena of platelets and endothelial cells. Note that pericytes are present around involutive vessels. E. Only pericyte-like cells (arrows) forming small residual groups persist. A and B: Semithin sections. Toluidine blue. C-E: Ultrathin sections. Uranyl acetate and lead citrate. A, B, x 350; C-F, x 12000
molecules and proteolytic enzymes. Likewise, the behaviour of pericytes is controlled and may be modified by these regulatory mechanisms (Diagram 1).

The role of pericycle/EC interactions in these mechanisms has been amply demonstrated by means of cocultures of ECs and pericytes (Gitlin and D’Amore, 1983), using mouse mutants and by antibody blocking and gain-of-function studies. Indeed, cell-cell contacts and autocrine and paracrine actuation of multiple diffusible soluble factors produced by pericytes and ECs participate in their bidirectional relationship, interdependence, position, number, phenotype, mutual survival and functions, as well as in the maintenance of vascular integrity and stability in quiescent microvasculature. Quiescent stage associations and interactions are transitorily difined (undergo molecular changes -Berger et al, 2005-) during vascular system development, maturation and remodelling, and subsequently restored. These modified interactions mediate and control pericycle and EC activation, morphology, metabolism, motility (migration), proliferation, homing, differentiation, release of several signals and matrix deposition, both regarding inhibition or stimulation (Newcomb and Herman, 1993; Jain, 2003; Armulik et al., 2006; von Tell et al., 2006; Hall, 2006; Motiejunaité and Kazlauskas, 2008). Nevertheless, “in vivo”, there are other components that also modify the response, including the following: a) different recruited blood cells and corpuscles, such as mononuclear cells, granulocytes and platelets, b) tissue specific vascular growth factors, c) extracellular matrix and d) plasma molecules. All these components determine the presence in the tissue of clusters of soluble factors (growth factors, cytokines and other factors), originating a complex network of interactions (Diagram 1).

The molecules and factors that play pivotal roles in the different aspects of ECs and pericytes during quiescent and angiogenic stages of the vasculature (Lindahl et al., 1997; Gerhardt et al., 2000; Nishishita et al., 2004; Nishishita and Lin, 2004; Armulik et al., 2005; Hoffmann et al., 2005; Garmy-Susini et al., 2005; Lehti et al., 2005; Foo et al., 2006; Kurup et al., 2006; Abramsson et al., 2007) can be grouped according to their type, source or most important action in vascular regulation. We have chosen the latter option, including its drawbacks (e.g. the same factor may have different, even opposing roles, depending on its spatial and temporal concentration profiles), following a similar sequence to that used in the section for pericycle behaviour during quiescent and angiogenic stages of blood vessels, as this allows for orderly, understandable and dynamic exposure.

**Principal factors and molecules that participate in pericycle/EC dissociation, migration and proliferation during early angiogenesis (in parent vessels and in sprout formation).** As described above, during early angiogenesis the parent vessels dilate and become leaky. Furthermore, their basement membrane locally dissolves. After dissociation, pericyte and ECs migrate and proliferate with sprout formation. Pericytes also originate transitional cell forms with myofibroblasts in the interstitium. Tissue hypoxia is one of the major triggers of these phenomena. Nitric oxide stimulates vasodilatation. VEGF increases permeability. Both activation of proteases and suppression of protease inhibitors dissolve basement membrane and extracellular matrix. Angiopoietin 2 can function inducing loosening of the pericyte/EC associations. Plasma proteins contribute to form a provisional matrix. Interactions between integrins and extracellular matrix facilitate EC and pericycle migration. Mitogens, including VEGF, FGF and PDGF participate in EC and pericycle proliferation. Other molecules, such as NG2 and endothelin-1, promote and mediate some of the above mentioned actions. Angiopoietin 2 in presence of VEGF facilitates sprout formation. These factors are outlined below in the order described, with the exception of PDGF, which will be addressed in the section on principal factors and molecules in recruitment of pericytes (Diagram 1).

Nitric oxide (NO). In blood vessels, endothelial nitric oxide synthase (eNOS) generates NO that, among other functions, mediates regulation of blood vessel tone (vasodilatation), flow and permeability, as well as neovascularization with mural cell recruitment to immature angiogenic sprouts (Yu et al., 2005). NO modulates the effects of several angiogenic factors (Duda et al., 2004). Thus, eNOS plays an important role in VEGF induced permeability and angiogenesis (Fukumura et al., 2001). Intervention in pericycle recruitment may be due to a marked increase in PDGF-β receptor gene expression (Yu et al., 2005) (see below).

Vascular endothelial growth factor (VEGF). VEGF-β, initially purified for its capacity to induce vascular permeability, is secreted, among other cells, by pericytes (presence of mRNA for VEGF -Tuder et al., 1995) above all under hypoxic conditions (Yamagishi et al., 1999). In repair through granulation tissue, macrophages, fibroblast-like cells, keratinocytes and other tissue cells are a source of VEGF (Jain, 2003). The VEGF-β receptors, VEGFR-1 (FLT-1), VEGFR-2 (KDR or FLK-1) and VEGFR-3 (FLT-3) (Dumont et al., 1995) and accessory receptors ( neuropilins), mediate permeability and growth actions (in particular, VEGFR-2 stimulates and VEGFR-1 has a negative role). VEGF is fundamental to initiate the formation of immature vessels during development and in adult life (Yancopoulos et al., 2000), since VEGF and VEGF receptors are the first S-specific signal transduction pathways activated (Ferrara et al., 2003; Ribatti et al., 2007). Indeed, during angiogenesis, VEGF is expressed in higher levels (VEGF mRNA is upregulated in pericytes) (Kim et al., 1998; Ferrara, 2004; Hoeben et al., 2004; Ribatti et al., 2005), and the levels of VEGF receptor also increase in growing vascular sprouts (Ferrara et al., 2003). After vessel stabilization, the
expression of VEGF decreases to basal levels. By increasing permeability, VEGF facilitates the formation of a provisional matrix and, activating EC migration and proliferation, induces formation and sprouting of capillary EC. In this way, using a 3D “in-vitro” model of angiogenesis, Ramsauer and D’Amore (2007) observed that VEGF, acting as a mitogen, was the only factor that induced capillary EC-like structures in bovine retinal capillary ECs. Besides, VEGF upregulates proteases, induces EC specialization and suppresses apoptosis (Jain, 2003). These roles of VEGF can act together with those of other factors (Yancopoulos et al., 2000). Soluble forms of VEGFR-1 and VEGFR-2 inhibit VEGF induced angiogenesis and have a direct effect on pericytes, promoting their migration (Lorquet et al., 2008).

VEGF-A, through the activation of VEGFR-1 and VEGFR-2, and MAPK/ERK and PBK/AKT pathways, induces increased permeability of the pre-existing microvasculature (Witmer et al., 2004) and activates EC and pericyte proliferation and migration (Yamagishi et al., 1999). VEGFA acts directly on pericytes, since...
several studies have shown that pericytes express VEGFER-1 (Takagi et al., 1996; Witmer et al., 2004).

Protease/antiprotease balance and components released from extracellular matrix. Activation of proteases, such as matrix metalloproteinases (zinc-dependent enzymes) (MMP2, MMP3 and MMP9) and urokinase plasminogen activator, and suppression of protease inhibitors, such as tissue inhibitor of proteinases and plasminogen activator inhibitor 1 (PAI1), intervene in proteolitic processing of basement membrane and surrounding interstitial matrix (Jain, 2003; Johnson et al., 2004), with release of growth factors (FGF2, VEGF, TGF-β and PDGF-β) stored in their heparin-like glycosaminoglycans. In this way, MMP-2 production by pericytes and ECs has been associated with vessel sprouting (Virgintino et al., 2007). Inflammatory and tumor cells also secrete proteinases and proteinase inhibitors (Mignatti and Rifkin, 1996).

Plasma components. Plasma components, including thrombin and angiotensin II, participate in quiescent and angiogenic stages of blood vessels. Likewise, plasma proteins, such as fibrinogen and fibronectin, contribute to form a provisional matrix that interacts with the migrating ECs and pericytes.

Integrins. Integrins (a family of noncovalently associated heterodimeric transmembrane glycoprotein adhesion molecules) expressed by pericytes include receptors for laminin (α3β1, α6β1, α6β4 and α7β1), collagen (α1β1, α2β1), fibronectin (α4β1, α5β1) and osteopontin (α9β1; α8β1) (Silva et al., 2008). These integrins, except α7β1 and α8β1, and others, vitronectin receptors αvβ3 and αvβ5, are expressed by ECs. The changes in cell adhesion during angiogenesis require upregulation of specific integrins, such as the receptors for fibronectin, collagen and vitronectin (Silva et al., 2008).

Basic fibroblast growth factor 2 (FGF2). FGF2, a heparin-binding polypeptide, is secreted by pericytes. FGF2 interacts with tyrosine kinase-FGF receptors of ECs. In this way, pericytes stimulate “in vivo” angiogenesis of EC through secretion of FGF-like molecules (Watanabe et al., 1997; Morisaki et al., 1999). In vivo, FGF2 induces angiogenesis and in vitro stimulates protease production and EC migration and proliferation (Basilico and Moscatelli, 1992; Ribatti et al., 2007). FGFs are potent mitogens (Montesano et al., 1986) and FGF2 enhance “in vitro” capillary formation in the presence of VEGF, acting primarily as a mitogen (Ramsauer and D’Amore, 2007).

NG2. Pericytes express the NG2 proteoglycan during the early stages of angiogenesis, and soluble NG2 promotes EC motility and angiogenesis via engagement of galactin-3 and α3β1 integrin (Fukuhsi et al., 2004). Furthermore, NG2 acts by binding with basic fibroblast growth factor BFGF, PDGF-AA, the Kringle domains of plasminogen and angiostatin (Ozerdem and Stallcup, 2004).

Endothelin-1. Endothelin-1, a mediator of pericyte-EC interactions produced by ECs, mediates proliferation of pericytes (mitogen) (Yamagishi et al., 1993) and vascular contraction (constrictor) (Chakravarthy and Gardiner, 1999).

Angiopoietin 2. Ang 2, expressed by ECs of sprouting or regressing vessels, has been considered a natural antagonist for Tie2 (Maisonpierre et al., 1997), although it may either activate or antagonize Tie2 (Maisonpierre et al., 1997), destabilizing the signal involved in initiating angiogenic remodelling (Maisonpierre et al., 1997; Goede et al., 1998; Holash et al., 1998; Yancopoulos et al., 2000; Vajkoczy et al., 2002; Hammes et al., 2004). Therefore, Ang 2 can function to loosen pericyte-EC interactions (Maisonpierre et al., 1997) and may contribute to revert vessel cells to a more plastic and destabilized state, facilitating angiogenesis (vessel sprouting and intussusception) in the presence of angiogenic growth factors (e.g. VEGF) or vascular regression in the absence of these growth factors (Dumont et al., 1994; Sato et al., 1995; Maisonpierre et al., 1997; Asahara et al., 1998; Suri et al., 1998; Thurston et al., 2000; Yancopoulos et al., 2000; Loughna and Sato, 2001; Vajkoczy et al., 2002; Hammes et al., 2004). In this way, Ang 2 causes pericyte dropout in retina, with the development of vascular capillaries (Hammes et al., 2004). The balance between Ang 1/Ang 2 conditions vascular stability/destability (see below). During early stages of angiogenesis, PDGF-β decreases Ang1/Ang2 ratio, promoting vessel destabilization (Nishishita and Lin, 2004).

Factors and molecules that participate in modifications of ECs and of transitional cell forms between pericytes and other cells during vessel regression. EC apoptosis and intravascular thrombi are observed during vessel regression, especially in some forms of excessive angiogenesis. As described above, angiopoietin 2 reverts vessel cells to a de-stabilized state and, in the absence of growth factors (e.g. VEGF), facilitates vascular regression, with EC apoptosis. Likewise, during the massive regression of most of the vessels created in the repair through granulation tissue, we have pointed out the importance of the marked accumulation of platelets (platelet thrombi) in the involutive vessels (Fig. 7), converting the granulation tissue in a “paracrine organ” with release of platelet factors (see below). These platelet factors, together with those generated by other cells, can increase the migration, proliferation, recruitment and differentiation of the transitional cell forms between pericytes and newly recruited mural cells, myofibroblasts, other interstitial matrix forming cells and preadipocytes (Díaz-Flores et al., 2009).
Principal factors and molecules in recruitment of pericytes, in pericyte differentiation and in pericyte/EC maturation during vessel stabilization and remodeling. During this phase, the following occur: recruitment of pericytes (migration and proliferation are associated findings), mesenchymal cell differentiation with pericyte/EC maturation and contact formation, extracellular matrix production, stabilization, tissue-specific specialization and arterio-venous determination. The pathways PDGF-PDGFR (related to extracellular matrix degradation), and sphingosin-1-phosphate1-EDG1 participate in recruitment of pericytes (PDGF-PDGFR promotes pericyte migration and proliferation, and S1P-EDG1 pericyte migration). TGF-β-TGF-beta RI (ALK-5, ALK-1), TGF-β RI and RgS5 induce mesenchymal cell differentiation and extracellular matrix formation. Angiopoietin 1-Tie 2 receptor tyrosine kinase promotes pericyte/EC maturation and communication, cell-ECM interactions and release of HB-EGF (heparin-binding epidermal growth factor-like growth factor) that also stimulates cell migration and proliferation. N-cadherin participates in leukocyte/EC contact formation. Integrins facilitate stabilization. MMPs may also contribute, directly or indirectly, to pericyte recruitment (Chantrain et al., 2006). Local factors (e.g., endocrine gland derived EG-VEGF) induce tissue-specific specialization (e.g., EC fenestration) and Ephrins-Eph receptors and the Notch pathway participate in arterio-venous determination.

Platelet-derived growth factor (PDGF). PDGF-β is secreted, among other cells, by ECs, probably in response to VEGF (Ribatti et al., 2007), and PDGF-β is expressed by mural cells (pericytes and VSMC) (Lindahl et al., 1997; Hellstrom et al., 1999), with interaction between ECs/mural cells as a paracrine signal (Holmgen et al., 1991; Crosby et al., 1998; Enge et al., 2002). The other sources of PDGF include the mural cells themselves, macrophages and platelets. During development, expression of PDGF-β is restricted to arteriolar and capillary EC (veins only have rudimentary SMC at this time) (Hellstrom et al., 1999). The production of PDGF is upregulated by sprouting angiogenesis (PDGF-β expression is concentrated at the tip of the sprouts -Armulk et al., 2005-) and elevated shear stress. PDGF-β binds to the heparan sulphate proteoglycans via matrix-retention motifs, a short stretch of basic aminoacids (in the C-terminus of the mature growth factor). This union mostly occurs in close vicinity to EC (LaRochelle et al., 1991) (in the extracellular matrix surrounding cells, near the production site, like many growth factors, cytokines and morphogens), where it is recognized by PDGF-β expressing pericytes (importance of local production and retention of the PDGF ligand to limit its range of influence and to guide correct orientation during migration and contact of pericytes) (Eming et al., 1999; Abramsson et al., 2003; Lindblom et al., 2003; Hall, 2006). PDGFR-β, stimulated by endothelial cell PDGF-β, undergoes autophosphorylation, recruits a diverse set of 10 SH2 domains and activates downstream cascades. In this way, during angiogenesis, PDGF-β acts as a paracrine factor and induces the following effects in the pericytes: a) proliferation (to expand the pericyte pool according to the levels of PDGF-β) and migration along the advancing EC sprouts (longitudinal recruitment -Armulk et al., 2005), (PDGFR mediate the chemotactic response -Westermark et al.,1990), b) recruitment of PDGFR-β + precursors cells and attachment to vessel walls (Rhodin and Fujita, 1989; Hirschi et al., 1998; Hellstrom et al., 1999; Abramsson et al., 2003; Kurup et al., 2006) (during angiogenesis, PDGF-β and its ligands are required for pericyte recruitment with failure in PDGF-β gene mutation and when PDGFR-β function is blocked by antibodies (Uemura et al., 2002; Leveen et al., 1994; Soriano, 1994; Lindahl et al., 1997)), c) differentiation and maturation with phenotype changes, emission of cell processes around ECs and establishment of contacts between them. Furthermore, during later stages of angiogenesis (vessel stabilization), PDGF-β upregulates release of TGF-β (via the MAPK/MEX pathway) and Ang1 (via P13Kinase/PKC pathway) (Nishishita and Lin, 2004) and downregulates release of Ang2 (Phelps et al., 2005). In turn, TGF-β and angio1 together decrease the release of PDGF-β by the endothelial cells. In conclusion, PDGF-β and its receptor play a key role in regulating pericyte/EC interactions and morphofunctional stability of vasculature (Betsholtz, 2004).

Components released from plasma and extracellular matrix. As we have stated above, heparan sulphate proteoglycans, present in the extracellular matrix surrounding cells and in the cell surface, control retention of some growth factors, including PDGF-β 1 (Kurup et al., 2006; Abramsson et al., 2007). Consequently, the sulphation degree of heparan sulphate proteoglycans also acts in the directional migration, recruitment and attachment of pericytes along sprouting vessels (Kurup et al., 2006; Abramsson et al., 2007). Therefore, metalloproteinases form part of the mechanism of pericyte recruitment (Chantrain et al., 2006). Thus, MT-1 MMP-PDGF receptor-beta axis regulates mural cell investment of the microvasculature (Lehti et al., 2005). In fact, the catalytic activity of MT1-MMP enhances PDGF-β signaling through PDGFR-β. On the contrary, circulating tissue-localized inhibitors of matrix metalloproteinases (TIMPs) act to prevent degradation of the provisional matrix around nascent vessels (Brew et al., 2000). Likewise, TGF-β1-induced ALK-5 signaling stimulates plasminogen activator inhibitor (PAI) in ECs (Hallman et al., 2005). In addition, fragments released by action of proteases, such as C-terminal hemopexin-like domain (PEX) from MMP2, angiostatin from plasminogen and tumstatin from collagen IV, behave as antiangiogenic molecules.
Sma and in its absence NG2 induced (Goumans et al., 2002, 2003). In presence of dependent manner, according to the signal cascades differentiation (Dickson et al., 1995) in a context migration, recruitment by induction, proliferation and 1997; Chambers et al., 2003) and modulates EC VSMC) from precursor mesenchymal cells (Pepper, induces the differentiation of mural cells (pericytes and 46x174) TGF-ß phosphorylation (Goumans et al., 2003). TGF-ß has phosphorylation, and ALK-5, with Smad 2/3 receptor pathways: ALK-1, with Smad 1/5 Itoh et al., 2000). Indeed, TGF-ß activates two types of specific target genes transcription (Derynck et al., 1998; 5 and ALK-1 (actin receptor-like kinase 5 and 1), which receptor that phosphorylates the TGF-ß receptors, ALK- 1987, 1988). In ECs, TGF-ß binds to active TGF-ß II (Ordlidge and D'Amore, 1987; Antonelli-Orlidge et al., 1989; Sato et al., 1990). Indeed, ECs and pericytes cultured separately both secrete latent TGF-ß, while cocultures produce TGF-ß only in an active form, indicating that contact or close proximity between the cells is essential for the generation of activated TGF-ß (Ordlidge and D’Amore, 1988). Gap-junctions appear to be the contact that is most involved in this process (Hirschi et al., 2003). Immunoadsorption of coculture-derived conditioned media with antibodies for TGF-ß eliminates inhibitory activity (Ordlidge and D’Amore, 1987, 1988). In ECs, TGF-ß binds to active TGF-ß II receptor that phosphorylates the TGF-ß receptors, ALK- 5 and ALK-1 (actin receptor-like kinase 5 and 1), which transduce the signal to the nucleus by phosphorylating specific Smad proteins, contributing to the regulation of specific target genes transcription (Derynck et al., 1998; Itoh et al., 2000). Indeed, TGF-ß activates two types of receptor pathways: ALK-1, with Smad 1/5 phosphorylation, and ALK-5, with Smad 2/3 phosphorylation (Goumans et al., 2003). TGF-ß has several roles in the vasculature. In this way, TGF-ß induces the differentiation of mural cells (pericytes and VSMC) from precursor mesenchymal cells (Pepper, 1997; Chambers et al., 2003) and modulates EC migration, recruitment by induction, proliferation and differentiation (Dickson et al., 1995) in a context dependent manner, according to the signal cascades induced (Goumans et al., 2002, 2003). In presence of TGF-ß, pericytes express αSMA and in its absence NG2 and desmin (Song et al., 2005) Furthermore, TGF-ß induces extracellular matrix deposition and an inhibitory effect on degradation of the provisional matrix in sprouting vessels by inducing plaminogen activator inhibitor 1 in ECs (Chambers et al., 2003) (A key factor in repair and in fibrogenic disorders). In a 3D “in vitro” model of angiogenesis, the addition of TGF-ß1 along with VEGF and FGF2 reduced or suppressed capillary-like structure formation (Ramsauer and D’Amore, 2007). Although many of these functions have been involved in controlling the resolution phase of angiogenesis (Pepper, 1997) and in stabilizing the endothelium by inhibiting EC migration and proliferation, stimulatory effects on angiogenesis must also be considered (TGF-β may be either pro or antiangiogenic) (Goumans et al., 2003). Indeed, depending on TGF-ß concentration and on expression of ALK-1 (mainly at sites of angiogenesis) and the number of activated signals transduction pathways, different effects may be produced (Tallquist et al., 2003). Thus, a low concentration of TGF-ß enhances VEGF or bFGF induced migration and invasion, while a high concentration of TGF-ß is inhibitory (Goumans et al., 2003). In this way, activation of ALK-5 inhibits EC migration and proliferation, while activation of ALK-1 stimulates these responses (Goumans et al., 2002, 2003). Proliferating ECs and pericytes/fibroblasts also express endoglin, a transmembrane accessory receptor for TGF-ß, required for efficient TGF-ß/ALK-1 signaling, promoting EC proliferation and reducing TGF-ß/ALK-5 signaling (Lebrin et al., 2004). Monocyte chemo-attractant protein-1 (MCP-1) mediates TGF-ß induced angiogenesis by stimulating mural cell migration (Ma et al., 2007).

Transforming growth factor (TGF). TGF-ß, a multifunctional cytokine that forms part of the superfamily of proteins, including activins and bone morphogenetic proteins (Piek et al., 1999), is produced by a number of cell types, such as pericytes and ECs, in a latent form, and collaboration of these cells by means of direct cell-cell contact is required for activation of latent TGF-ß (Ordlidge and D’Amore, 1987; Antonelli-Orlidge et al., 1989; Sato et al., 1990). Indeed, ECs and pericytes cultured separately both secrete latent TGF-ß, while cocultures produce TGF-ß only in an active form, indicating that contact or close proximity between the cells is essential for the generation of activated TGF-ß (Ordlidge and D’Amore, 1988). Gap-junctions appear to be the contact that is most involved in this process (Hirschi et al., 2003). Immunoadsorption of coculture-derived conditioned media with antibodies for TGF-ß eliminates inhibitory activity (Ordlidge and D’Amore, 1987, 1988). In ECs, TGF-ß binds to active TGF-ß II receptor that phosphorylates the TGF-ß receptors, ALK- 5 and ALK-1 (actin receptor-like kinase 5 and 1), which transduce the signal to the nucleus by phosphorylating specific Smad proteins, contributing to the regulation of specific target genes transcription (Derynck et al., 1998; Itoh et al., 2000). Indeed, TGF-ß activates two types of receptor pathways: ALK-1, with Smad 1/5 phosphorylation, and ALK-5, with Smad 2/3 phosphorylation (Goumans et al., 2003). TGF-ß has several roles in the vasculature. In this way, TGF-ß induces the differentiation of mural cells (pericytes and VSMC) from precursor mesenchymal cells (Pepper, 1997; Chambers et al., 2003) and modulates EC migration, recruitment by induction, proliferation and differentiation (Dickson et al., 1995) in a context dependent manner, according to the signal cascades induced (Goumans et al., 2002, 2003). In presence of TGF-ß, pericytes express αSMA and in its absence NG2 and desmin (Song et al., 2005) Furthermore, TGF-ß induces extracellular matrix deposition and an inhibitory effect on degradation of the provisional matrix in sprouting vessels by inducing plaminogen activator inhibitor 1 in ECs (Chambers et al., 2003) (A key factor in repair and in fibrogenic disorders). In a 3D “in vitro” model of angiogenesis, the addition of TGF-ß1 along with VEGF and FGF2 reduced or suppressed capillary-like structure formation (Ramsauer and D’Amore, 2007). Although many of these functions have been involved in controlling the resolution phase of angiogenesis (Pepper, 1997) and in stabilizing the endothelium by inhibiting EC migration and proliferation, stimulatory effects on angiogenesis must also be considered (TGF-β may be either pro or antiangiogenic) (Goumans et al., 2003). Indeed, depending on TGF-ß concentration and on expression of ALK-1 (mainly at sites of angiogenesis) and the number of activated signals transduction pathways, different effects may be produced (Tallquist et al., 2003). Thus, a low concentration of TGF-ß enhances VEGF or bFGF induced migration and invasion, while a high concentration of TGF-ß is inhibitory (Goumans et al., 2003). In this way, activation of ALK-5 inhibits EC migration and proliferation, while activation of ALK-1 stimulates these responses (Goumans et al., 2002, 2003). Proliferating ECs and pericytes/fibroblasts also express endoglin, a transmembrane accessory receptor for TGF-ß, required for efficient TGF-ß/ALK-1 signaling, promoting EC proliferation and reducing TGF-ß/ALK-5 signaling (Lebrin et al., 2004). Monocyte chemo-attractant protein-1 (MCP-1) mediates TGF-ß induced angiogenesis by stimulating mural cell migration (Ma et al., 2007).

Rgs5. Rgs5, expressed by pericytes, stimulates GTPase activity and subsequently inhibits signalling downstream of G-protein-coupled receptor (see above) and participates in the differentiation of vascular mural cells (Cho et al., 2003; Li et al., 2004).

Angiopoietin 1. Angiopoietin 1, ligand for the Tie 2 receptor, regulates vessel stability. Ang 1 is required for remodelling, maturation, stabilization and quiescence of the immature vasculature (Suri et al., 1996; Loughna and Sato, 2001) and can originate a circumferential format as opposed to sproutive growth (Valenzuela et al., 1999; Yancopoulos et al., 2000; Loughna and Sato, 2001). Ang 1, is produced by the pericytes and smooth muscle cells (Sundberg et al., 2002) and, functioning as a paracrine signal, activates the endothelial receptor tyrosine kinase Tie-2 (the receptor is also expressed in other cells such as proangiogenic monocyte/macrophage and mesenchymal pericyte progenitors (De Palma et al., 2005) and maximizes interactions between EC and pericytes (makes vessels leak resistant), enabling them to receive other signals from their environment (e.g. pericyte recruitment factor-PDGF- and hepatocyte growth factor- HGF from ECs) (Suri et al., 1996). Therefore, Ang 1 is involved in vessel diameter and permeability regulation (vascular permeability reduction) as well as in vessel maturation (Thurston et al., 2000; Uemura et al., 2002) and stabilization by promoting pericyte recruitment (Asahara et al., 1988; Hawighorst et al., 2002; Gerhardt and Betsholtz, 2003, Stoesselzinger et al., 2003). The effect of recruitment may be
indirect (e.g. stimulating HGF expression by EC to increase pericyte motility) (Kobayashi et al., 2006). Since Ang 2 favours vessel destabilization (see above) and angiopoietins 1 and 2 bind to the same receptor (Tie-2), the balance Ang1/Ang2 conditions vessel stabilization/destabilization.

N-cadherin. N-cadherin is expressed by both pericytes and ECs and mediates pericyte-EC interactions and regulates pericyte attachment through adhesion junctions (Gerhardt et al., 2000).

Integrins. α4-β1 integrin, expressed by ECs, and VCAM, expressed by pericytes, act in EC-pericyte adhesion and to ensure cell survival and patterning (Garmy-Susini et al., 2005). Taking into account what is stated in the previous sections of integrins, angiogenic sprouting and postangiogenic neovessel survival in collagen depend on β1 integrins, whereas survival in fibrin is controlled by β1 and β3 integrins (Carnevale et al., 2007).

Ephrins and Eph receptors. Some ephrin receptor kinases and their cell-surface-anchored ephrin ligands, which are expressed in arterial ECs (predominantly ephrins-B2), a cell surface-surface-anchored ligand and in veins (predominantly the receptor Eph B4) (Wang et al., 1998; Adams et al., 1999; Gerety et al., 1999), act in the control of arterial-venous blood vessel identity (Adams, 2003). Likewise, Ephrin B2 is a critical regulator of mural cell migration, spreading adhesion during blood-vessel wall assembly (Foo et al., 2006).

Notch. Notch signaling, which acts in arterial-venous differentiation during embryonic vascular development (regulated by genetic predisposition involving Notch signaling) (Lawson et al., 2001; Claxton and Fruttiger, 2004; Armulik et al., 2005), operates through local cell-cell interactivity and its actions in ECs and pericytes are as follows (Sainson and Harris, 2008): a) regulates pericyte recruitment and differentiation, b) may antagonize EC proliferation (among other possibilities, DLL4/Notch signaling produces transcriptional inhibition of VEGFR2, and Notch signaling induces the expression of soluble VEGFR1), and c) regulates vessel branching through tip cell versus stalk cell differentiation (Sainson et al., 2005). In dental pulp, perivascular cells mainly express Notch 3, whereas Notch 2 shows extensive expression and Notch 1 is manifested in cells close to lesions (Mitsiadis et al., 2003; Lovschall et al., 2005). Notch 3 expression in pericytes of the retina has also been demonstrated (Claxton and Fruttiger, 2004).

Physical interactions. Physical interactions through direct pericyte/EC contact act by providing mechanical support, reinforced by the pericyte processes (Rhodin, 1968; Forbes et al., 1977; Furchgott and Zawadzki, 1980) and by activating latent factors in the extracellular matrix (e.g. latent transforming factor B) (see pericycle contacts).

Interactions of pericytes and EC with recruited blood cells and other tissue cells

Diapedesis of leukocytes (neutrophils, macrophages and lymphocytes) precedes and accompanies angiogenesis during repair. The transmigration of these cells through blood vessel walls is not passive, since interactions between ECs, pericytes and migrating cells participate, by means of a cascade of signalling events, in different regulatory mechanisms, including those mentioned above. Thus, neutrophils are a source of VEGF-A and Interleukin-8 (Li et al., 2003; Ohki et al., 2005; Schruefer et al., 2006). macrophages for Interleukin-1, TNFα, PDGF-AB, TGFβ and FGF-β, VEGF, prostaglandin and reactive oxygen species (Polverini et al., 1977; DiPietro and Polverini, 1993; Moldovan and Moldovan, 2005). T-lymphocytes produce interferon gamma, Interleukin-1 and TNF-α. Besides, platelets contribute with PDGF-AB, TGF-β, epidermal growth factor EGF/tumor necrosis growth factor alpha, tromboxane and insulin-like growth factor.

During the initial phase of angiogenesis, the migrating monocyte/macrophages may also contribute to the dissociation and detachment of the activated pericytes in parent vessels before vascular sprouts. Indeed, we have described morphologic association between monocyte/macrophages and pericytes during this stage. This association continues when the pericytes are partially detached from the vessel wall, and when they acquire transitional cell forms with myofibroblasts, suggesting interactive cooperation in migration, differentiation and functional activity (like a tug-macrophage-towing a ship -pericyte-myofibroblast) (Figs. 4C, 5D) (see above) (Díaz-Flores et al., 2009).

In the CNS, it has been demonstrated that MMP-3, released by pericytes, activates pro MMP-9 produced by astrocytes (Rosenberg, 2005). Likewise, pericyte-EC interaction increases MMP-9 secretion at the blood-brain barrier in vitro (Zoulya et al., 2008).

In neovascularization, the formation of a basic vascular network is supported by extracellular matrix molecules, such as fibronectine, which decrease during vessel maturation, coinciding with the synthesis of laminin and collagen type IV (Drake et al., 1990).

Perivascular mesenchymal Stem cell niche hypothesis

Vasculature as a niche for adult stem cells

The adult stem cells (ASC) reside in a specialized physical location known as a niche (Schofield, 1978; Díaz-Flores et al., 2006), which constitutes a three-dimensional microenvironment containing, in addition to ASC, neighbouring differentiated cell types and extracellular matrix. Therefore, niches are specific
locations in a tissue where stem cells can reside for an indefinite period of time and produce progeny cells while self-renewing. The niche contributes a regulatory system, which maintains and governs the location, adhesiveness, retention, homing (recruiting) and mobilization, quiescence or activation, rate of division, orientation of mitotic axes, types of division (symmetric or asymmetric) and differentiation of the ASC. For example, symmetrical divisions may originate two ASCs, or two transit amplifying cells (TAC), and asymmetrical division one ASC and one TAC, controlling the number of ASC and TAC. Cell contacts with neighbouring cells (adherens junctions-cadherins and cateners) and cell adhesion molecules with extracellular matrix (integrins-basal membrane), and the balance of stimulatory and inhibitory signals that regulate cell quiescence (growth factor, mitogenic cytokines and WNTS signalling) participate in this regulatory mechanism.

The term “vascular niche” may be used as a broad concept (vascular niche in general) or as a more strict and particular aspect: multipotent perivascular mesenchymal stromal cell (MSC) niche (the subject of this section).

The broad concept considers the vascular niche as a site especially rich in blood vessels where ECs and mural cells (pericytes and SMC) create a microenvironment that affects the behaviour of several stem and progenitor cells (Palmer et al., 2000; Louisaint et al., 2002; Carmeliet, 2003b; Nikolova and Lammert, 2003; Aveilla et al., 2004; Wurmser et al., 2004; Kiel et al., 2005; Kopp et al., 2005; Ramírez-Castillejo et al., 2006; Nikolova et al., 2007). For example, neural stem cell niche in the adult subventricular zone contains an extensive planar vascular plexus (Shen et al., 2008; Tavazoie et al., 2008), and the stem cells and their transit-amplifying progeny contact the vasculature at sites that lack astrocyte endfeet and pericyte coverage (Tavazoie et al., 2008). Thus, it has been proposed that vascular niches share the ability to provide a basement membrane to cells with high plasticity, which are unable to form their own (they are not usually part of a polarized tissue with a stable basement membrane) such as budding epithelial cells, neural stem cells, proliferating haematopoietic stem cells, pancreatic β cells and metastatic cancer cells (Nikolova et al., 2007). Therefore, the vascular basement membrane is considered a key part of all vascular niches (Nikolova et al., 2007), with the following roles: a) providing support for proliferating and differentiating stem and progenitor cells, b) increasing signalling by growth factors, as occurs for haematopoietic stem cells (factor 4 -FGF4- and stromal cell derived factor 1 -SDF1- -Kopp et al., 2005), c) facilitating cell homing and migration (Even-Ram and Yamada, 2005), d) polarizing cells, enabling symmetric and asymmetric cell divisions, and e) binding proteins of basement membrane to integrins with signals on their own and amplifying the cell response to several growth factors.

As mentioned above, a particular aspect of the vascular niche is the perivascular niche for the MSCs, a hypothesis which was suggested some time ago (Díaz-Flores et al., 1991; Nehls et al., 1992) and which has been reinforced recently (Bianco et al., 2001; Gronthos et al., 2003; Shi and Gronthos, 2003; Bhalshki et al., 2006, 2008; Da Silva Meirelles et al., 2006, 2008; Djouad et al., 2007; Kolf et al., 2007; Sacchetti et al., 2007; Arthur et al., 2009; Covas et al., 2008; Crisan et al., 2008; Khan et al., 2008; Tare et al., 2008; Zannettino et al., 2008). This perivascular niche encompasses the MSCs, their neighbouring cells (immediately surrounding the MSCs), the extracellular matrix and the soluble molecules. Therefore, this niche constitutes a microenvironment where a subset of pericytes are protected and controlled in their self-renewing capacity and differentiation. In this regulatory system, pericyte/EC interactions play an important role. Likewise, the interactions of EC and pericytes with extracellular matrix, including the basement membrane, as well as with homing or transmigrating cells through paretial microvasculature should be taken into account. The secretion of autocoids, autocrines and paracines, as well as the mediators present in the microcirculation, participates in the mechanisms that intervene in the regulation of quiescent and angiogenic stages of blood vessels. Next, we consider the principal arguments that support the perivascular MSC niche hypothesis, that is, the pericytes and their ancestors (mesoangioblasts) as progenitor cells (similar plasticity to mesenchymal stem cells) and the findings that identify a perivascular location for MSCs.

**Pericyte function as a progenitor cell. Pericyte plasticity**

In resting tissues, pericytes and EC are quiescent-slow-cycling cells, while in the angiogenic stage, they show high proliferative potentiality and the ability to self-renew and to originate daughter cells. Therefore, these cells or subsets of these cells share findings with adult stem cells.

Pericytes are therefore considered by some authors as progenitor cells with great mesenchymal potential and as a source of undifferentiated mesenchymal cells (Fig. 8 and Diagram 2) (Díaz-Flores et al., 1991a,b, 1992b, 2006; Nehls and Drenkhan, 1991, 1993; Nehls et al., 1992; Sundberg et al., 1996; Doherty et al., 1998; Brachvogel et al 2005; Dellavalle et al., 2007). In postnatal life, retaining considerable mesenchymal potentiality, pericytes isolated from different tissues may have the capacity to differentiate into other cell types (Díaz-Flores et al., 1991a; Zannettino et al., 2008), such as fibroblasts/myofibroblasts (Ross et al., 1970, Ronnov-Jessen et al., 1995; Sundberg et al., 1996; Farrington-Rock et al., 2004), chondroblasts (Díaz-Flores et al., 1988), osteoblasts (Takahashi and Urist 1986; Schor et al., 1990; Díaz-Flores et al., 1992b; Ronnov-Jessen et al., 1995; Schor et al., 1995; Sundberg et al., 1996; Collett and Canfield, 2005; Hall, 2006), odontoblasts...
Pericytes and mesenchymal cell niche

Pericytes and mesenchymal cell niche

(Mesoangioblasts as vessel-associated stem cells

During development, mesoangioblasts are considered vessel-associated mesodermal stem cells (De Angelis et al., 1999; Minasi et al., 2002; Cosso and Bianco, 2003; Brunelli et al., 2004), which can be induced to differentiate into different cell types of the mesoderm, including smooth muscle cells, bone and cartilage (De Angelis et al., 1999; Minasi et al., 2002, Cosso and Bianco, 2003; Brunelli et al., 2004; Tagliafico et al., 2004). Bianco and Cosso (1999) proposed that mesoangioblasts, originating from the dorsal aorta and accompanying the angiogenic vessels, leave the latter and adopt the fate of the tissue where the vessels enter. This concept provides further support for a perivascular niche hypothesis, since mesoangioblasts may represent ancestors of postnatal stem cells, and some vessel-associated progenitors (pericytes or subset of pericytes) may remain undifferentiated and capable of differentiating during postnatal life (De Angelis et al., 1999; Minasi et al., 2002; Cosso and Bianco, 2003; Brunelli et al., 2004).

Identification of an MSC niche

Since pericytes have mesenchymal capacity (pericytes as multipotent progenitor cells, see above), the hypothesis of their participation in an appropriate MSC niche has been raised and is steadily gaining credibility. In addition, complementary studies have enhanced the concept that MSCs are located in perivascular sites of different tissues (Richardson et al., 1982; Schlondorff, 1987; Díaz-Flores et al., 1991a; Nehls et al., 1992, Bianco et al., 2001b, De Bari et al., 2001, Gronthos et al., 2003b, Shi and Gronthos, 2003; Wexler et al., 2003; Sabatini et al., 2005; Da Silva Meirelles et al., 2006; Dore-Duffy et al., 2006; Djouad et al., 2007; Lovschall et al., 2007; Sachetti et al., 2007; Covas et al., 2008; Traktuev et al., 2008; Zannettino et al., 2008). Indeed, MSCs have been isolated from numerous structures, such as bone marrow, periosteum and trabecular bone, muscle, adipose, tendon, brain, liver, spleen, kidney, thymus, lung, pancreas, heart, ovary, dermis, synovium, deciduous teeth (Barry and Murphy, 2004; Blashki et al., 2006; Da Silva Meirelles et al., 2006), and a perivascular niche for these cells has been pointed out (Brighton et al., 1992; Galmiche et al., 1993; Nehls and Drenckhahn, 1993; Schor et al., 1995; Doherty et al., 1998; Schor and Canfield, 1998; Carlile et al., 2000, Bianco et al., 2001b; Helmbold et al., 2001; Zuk et al., 2001; Gronthos et al., 2003; Shi and Gronthos, 2003; Brachvogel et al., 2005, 2007; Da Silva Meirelles et al., 2006; Djouad et al., 2007; Kolf et al., 2007; Sacchetti et al., 2007; Crisan et al., 2008; Covas et al., 2008; Da Silva Meirelles et al., 2008; Khan et al., 2008; Tare et al., 2008; Zannettino et al., 2008; Arthur et al., 2009; Díaz-Flores et al., 2009).

Some of the previous authors, despite not using the term “niche”, considered the microvasculature networks as a possible location and source of MSCs (Díaz-Flores et al., 1991; Nehls et al., 1992; Doherty et al., 1998; Tare et al., 2008). Likewise, Bianco et al., (2001) suggested that bone marrow stromal cells, with mesenchymal potential, were components of the vascular wall.

MSC expression of pericyte markers is among the complementary studies that suggest the perivascular niche hypothesis (Traktuev et al., 2008). In this way, stem cells expressing STRO1, isolated from human bone marrow and dental pulp, display phenotypes (positivity for αSM actin and CD146, and variable expression for the pericyte marker 3G-5), suggesting that two distinct primitive stem cell populations are intimately associated with the blood vessels of their respective tissues (Shi and Gronthos, 2003). Coexpression of RgS5 (a marker for...
pericytes) and Notch3 (Notch signalling pathways regulate stem cell fate specification and express in perivascular cells) have been observed in pericytes of developing and injured teeth, indicating the importance of pericytes as a source of stem cells during dental repair (Lovschall et al., 2007). Immunohistochemical expression of Sca-1+/Thy/1+/CD31+ cells in perivascular sites also suggests a perivascular location for the MSC niche (Blashki et al., 2006). Brachvogel et al. (2005, 2007) have pointed out that perivascular cells, expressing annexin A5 gene (a marker for perivascular cells expressed during early stages of vasculogenesis), isolated from mouse tissues (embryos and adult brain meninges), show unique expression profiles of markers characteristic of pericytes and MSCs (NG2, SM actin protein, PDGFR-β, FLK-1 kit, Sca 1, CD-34), whereas endothelial (PECAM), haematopoietic (CD-45) or myeloid (F4/80, CD-11B) lineage markers were not detectable. Furthermore, the isolated perivascular cells had the capacity to differentiate into mesenchymal stem cell lineages (adipose and osteoblastic cells) and also displayed phagocytic activity (Brachvogel et al., 2005, 2007). Self-renewal capacity and osteogenic and adipogenic potentiality were demonstrated using cultures originated from glomerular mesangial cells (which are considered specialized pericytes—see below). In this experiment and on the basis of αSMA expression in MSCs isolated from all tissue types tested, the authors concluded that the MSC compartment extends through the whole post-natal organism as a result of its perivascular location (Da Silva Meirelles et al., 2006). In the same way, human infrapatellar fat pad-derived stem cells expressing the pericyte marker 3G5 showed enhanced chondrogenesis after expansion in fibroblast growth factor 2 (Khan et al., 2008). Using markers associated with mesenchymal and perivascular stem cells STRO-1, CD-146 and 3G5, a multipotent cell population within adult human adipose tissue was described also exhibiting a perivascular phenotype by Zannettino et al. (2007). The demonstration of a linear correlation between the numbers of adipose stem cells, obtained from both highly and poorly vascularised sites of equine adipose tissue, and vascular density is indicative of MSC physical association with blood vessels (Caplan, 2007; Da Silva Meirelles et al., 2008). Furthermore, freshly isolated stromal vascular fraction cells, expressing CD34, separated from CD31+, CD144+ EC, coexpress mesenchymal (CD10, CD13 and CD90), pericytic (chondroitin sulphate proteoglycan, CD140a and CD 140b) and smooth muscle (α–actin, caldesmon and calponin) markers, which enhance the hypothesis that considers the majority of adipose-derived adherent cells (with MSC properties) as resident pericytes (Traktuev et al., 2008). Nevertheless, none of the markers is specific or continually expressed by pericytes and descendent cells (see above). Likewise, the absence of MSC specific markers and their modification in cultures hinder MSC identification “in vivo” and “in vivo”.
Fig. 8. Demonstration of pericyte function as progenitor cell (Pericyte plasticity). A, B. Pericytes (P) marked by Monastral Blue (arrows) in the wall (A) and detaching from the wall (B) of postcapillary venules. EC: Endothelial cells. Monastral Blue is subsequently observed (arrows) in myofibroblasts (C and D, MF), chondroblasts (E-G, CH) and osteoblasts (H, I, O) after granulation tissue formation, and chondrogenic and osteogenic stimulation, respectively. OS: Osteoid. A-C, E, F, H: Semithin sections. Toluidine blue. D, G and I: Ultrathin sections. Uranyl acetate and lead citrate. A-C, E, F, x 450; C, G, I, x 15000; H, x 250
Pericytes and mesenchymal cell niche

Pericyte and EC “marriage and divorce”

Pericytes and ECs reside in a specialized physical location, the pericytic microvasculature, which constitutes a three-dimensional microenvironment, also including extracellular matrix and basement membrane components, as well as transmigrating cells. Intima association and bidirectional interactions between pericytes and ECs, along with microenvironment influences, contribute to the maintenance of vascular stability, with adequate pericyte/EC association (pericyte and EC “marriage”- see above). When this complex mechanism is altered, the microvasculature changes and becomes a substrate of a general inflammatory-reparative system (Díaz-Flores et al., 2009) in which, to a greater or lesser extent, the following findings may occur: a) vasodilation and increase of vascular permeability (leakage through the vessel wall), b) modulation of the extracellular matrix, c) recruitment of inflammatory cells, d) angiogenesis with new capillary formation, regression, remodelling and maintenance and d) contribution of matrix forming cells (fibroblasts-myofibroblasts, osteoblasts, chondroblasts), and contractile and adipose cells (Diagram 2). Together with these events, an important phenomenon is the initial and transient separation between pericytes and ECs (“divorce”) in the following steps: juxtaposed, partially dissociated and separated. The dissociation between pericytes and ECs is facilitated by upregulation expression of genes involved in vessel formation, such as VEGF, angiopoietin 2 and nitric oxide synthetase, as well as by activation of metalloproteinases and suppression of their inhibitors. Hypoxia triggers this mechanism, which also leads to vessel dilatation (nitric oxide), increased vascular permeability (VEGF) and the disintegration of vascular basement membrane and extracellular matrix (proteases). In these conditions, the ECs and pericytes rapidly pass from an associated, quiescent and stable state (“marriage” - into their perivascular mesenchymal niche) to a dissociated, mobile, proliferative and transitory situation (“divorce”), with initial formation of vascular sprouts (angiopoietin 2) and migration of some pericytes toward the interstitium wherein they behave as precursor cells (Diagram 2). Leaked plasma proteins serve as a provisional matrix where ECs and pericytes migrate through interactions between integrins and a provisional matrix. Several factors intervene in this migration and proliferation of ECs and pericytes. Descendants of the dissociated and migrating pericytes, behaving as highly proliferative cells (transit-amplifying cells), acquire the ability to differentiate into other pericytes, matrix forming cells, smooth muscle cells or adipocytes. The recruitment of pericytes and new association between ECs and pericytes (“new marriage”) are facilitated by several signaling pathways, principally PDGF-beta/PDGF-R-beta receptor, TGF-β 1/ALK-5, SIP/EDG-1 and ANG/Tie 2 (Jain and Booth, 2003). Furthermore, pericytes not only stabilise vessels but may also promote angiogenesis. For example, NG2 proteoglycan expressed by pericytes increases EC motility and cord angiogenesis (Fukushi et al., 2004) (see above).

Peculiar types of pericytes

In some capillaries where pericytes apparently do...
not exist, cells apposed to endothelial cells were shown to have some characteristics of pericytes (Fujimoto and Singer, 1987), such as hepatic stellate cells (Ito cells) in hepatic sinusoids, bone marrow and splenic sinusoid reticular cells and mesangial cells (Fig. 9). Other pericytes have particular properties such as occurs in CNS (blood barrier function), in cochlear stria vascularis and in some others.

**Hepatic stellate cells (Ito cells)**

Hepatic stellate cells (HSCs) (Ito cells, hepatic lipocytes or fat-storing cells) (Fig. 9A), characterized by vitamin A-containing lipidic droplets (retinoids: vitamin A and its metabolites), located in the space of Disse, are analogous to tissue pericytes (Pinzani et al., 1992; Pinzani, 1995; Saile et al., 1999; Sato et al., 2003; Lee, 2007), owing to the following facts: a) location in close contact with sinusoidal EC (between sinusoidal EC and parenchymal cell plates, with incomplete basement membrane and interstitial collagen fibers), b) similar ultrastructural features, c) capacity of reversible contraction on response to several vasoconstriction stimuli (induction of contraction by tromboxane A2 analogue, prostaglandin E2 (Kawada et al., 1992, 1993; Pinzani et al., 1992; Rockey et al., 1993; Sakamoto et al., 1993; Tran-Thi et al., 1993), d) expression of the αSMA gene during activation (Ramadori et al., 1990), desmin, NG2 and glial fibrillar acidic protein (Yokoi et al., 1984; Gard et al., 1985), e) role in angiogenesis and vascular remodelling, f) mitogenic and motogenic response to PDGF (Bachem et al., 1989; Pinzani et al., 1989), and g) TGF-β-dependent extracellular matrix regulation (Ramadori et al., 1987; Schafer et al., 1987; Davis, 1988; Maher et al., 1988; Arthur et al., 1989; Czaja et al., 1989). The embryonic origin (mesodermal, endodermal or neuroectodermal) of HSCs is as yet unresolved (Geerts, 2004). Bone marrow precursor cells have been considered as a possible source of HSCs (Baba et al., 2004; Suskind and Muench, 2004). During hepatic regeneration, migration of HSCs and ECs results in the formation of new sinusoidal branches (Lee et al., 2007) and HSC activation regulates sinusoidal structural changes (Issa et al., 2003; Kalinichenko et al., 2003; Balabaud et al., 2004; Mabuchi et al., 2004). HSCs show a stellate-shaped morphology with primary and secondary branches. The primary processes are long and run parallel to the sinusoids. The secondary branches partially encircle the sinusoids and penetrate between hepatocytes, and some of them reach neighbouring sinusoids. The cells store 80% of the total retinoids in the whole body (Senoo, 2004) (function of retinol transport and storage into liver - Blaner et al., 1985; Blomhoff, 1987; Hendriks et al., 1988), and in the liver HSCs are well established as collagen producing cells. In pathological conditions these cells lose their lipid droplets, acquire myofibroblastic phenotype (change their morphology from star to fusiform, enhance contractile properties, and their rough endoplasmic reticulum and Golgi apparatus become prominent), migrate, proliferate and increase the synthesis and secretion of extracellular matrix components (collagen, proteoglycan and glycoproteins) and metalloproteinases. Furthermore, HSCs release proinflammatory and promitogenic cytokines, participating in the recruitment of inflammatory cells in hepatic tissue repair (Knittel et al., 1999). Geerts (2007), has pointed out that neurotrophin receptor p75 (NTR), a tumor necrosis factor receptor superfamily member expressed in human HSCs after hepatic fibrosis and cirrhosis, is a regulator of repair and that HSC differentiation (supporting hepatocyte proliferation in the diseased liver) is impaired by inhibition of p75 (NTR signalling) for the guanosine triphosphatase Rho. A diffuse stellate cell system (with characteristics similar to HSCs) has been described in mammals (Nagy et al., 1997; Matano et al., 1999; Senoo, 2004; Zhao and Burt, 2007). This system contributes to the regulation of homeostasis of retinoids in the whole body (Senoo, 2004).

**Bone marrow reticular cells**

Present in the bone-marrow microvasculature (Fig. 9B) is a continuous layer of subendothelial pericytes (Andreeva et al., 1998), which acquires a reticular morphology (reticular cells) in the venous side. Therefore, the ECs in the highly permeable sinusoidal vessels are supported by a basement membrane, pericytes and reticular cells (Tavassoli, 1981; Nikolova et al., 2007), the latter emitting long processes into the haematopoietic cords (Bianco et al., 2001). In the arterial side of the microvasculature (arterial/capillary), pericytes express both alkaline phosphatase and αSMA, while the reticular cells in the venous side (postcapillary venous sinusoids) express alkaline phosphatase but not αSMA (Bianco et al., 2001). These reticular cells have the ability to convert into adipocytes. The marrow pericytes may be the same entity as the bone marrow stromal cells, since they share features such as: a) similar location of pericytes and stromal cells, b) expression of similar markers, such as SMA, PDGFR-β, EGFR and CD146, and c) similar response to growth factors (Bianco et al., 2001). Therefore, the bone marrow microvasculature networks have been hypothesized as one possible niche for MSCs (Doherty et al., 1998), coinciding with the concept that niches are highly vascularized sites (Spradling et al., 2001; Shi and Grontos, 2003; Tare et al., 2008). We have observed increased intracytoplasmic filaments in activated bone marrow stromal cells (Fig. 9B).

**Mesangial cells**

Mesangial cells are considered to be microvascular pericytes (Schlöndorff et al., 1987), which occur specifically in the renal glomeruli (30% of the glomerular cells). These cells and their surrounding matrix are located in a central position in the glomerulus,
Fig. 9. Peculiar types of pericytes. A. A hepatic stellate cell (Ito cell) (HSC), with lipidic droplets, is present in the space of Disse (H: Hepatocyte, EC: Endothelial cell). B. Activated bone marrow stromal cells (SC) next to haematopoietic cells (microfilaments -arrow- are present in one of the stromal cells). C. Mesangial cells (arrows) and surrounding matrix, located in a central position in the renal glomerulus. D. Pericytes (P) in blood-brain barrier. L: Vessel lumen, A: Astrocyte. Ultrathin sections. Uranyl acetate and lead citrate. A, B, x 15000; C, x 10000; D, x 13000
the mesangium (Fig. 9C). Mesangial cells are irregular in shape and show a dark-stained nuclei and short cytoplasmic processes, which slightly penetrate between the ECs and the glomerular basement membrane. The latter, along with the visceral epithelial cell layer, encircle the mesangium (mesangial cells and mesangial matrix) and the capillary loop surfaces without mesangial cells. In these zones, the podocytes can function as endothelial-supporting cells (Hirschberg et al., 2008). Mesangial cell processes establish direct and microfibril mediated attachment with glomerular basement membrane, acting as a biomechanical functional unit (Sakai and Kriz, 1987; Mundel et al., 1988; Kriz et al., 1990). The similarities between mesangial cells and pericytes (Schlöndorff et al., 1987; Tahara et al., 2006) include cell contractility, response to specific vasoactive molecules (e.g. endothelin 1; angiotensin 2 (Chansel et al., 1992; Rebibou et al., 1992), endocytosis (macromolecular uptake)), support for capillary loops, production and breakdown of basement membrane and extracellular matrix, and the same regulatory mechanisms. For example, PDGF is critical for development of mesangial cells, which participate increasing the capillary surface area in a single vascular loop by intussusceptive or splitting mechanisms (Betsholtz, 2004). Besides, mesangial cells in culture may have osteogenic and adipogenic differentiation capacities (Da Silva Meirelles et al., 2008) (see above). In this way, during development, mesangial cells are always continuous to the vascular pericyte-smooth muscle cells, and the findings suggest that mesangial cells differentiate from the primitive pericytes of the immature capillaries (Yamanaka, 1988). Finally, mesangial cells can respond to molecules as well as generate a variety of them (see Identification. Macromolecules expressed by pericytes and pericyte markers) and are involved in glomerular pathology.

Pericytes in CNS (Blood barrier function)

In addition to ECs, pericytes, astrocytes, microglia and extracellular matrix are essential components of the blood-brain barrier (Fig. 9D) (Farrell et al., 1987; Frey et al., 1991; Balabanov and Dore-Duffy, 1998; Alliot et al., 1999, Jäger et al., 1999; Lawrenson et al., 1999; Leino et al., 1999; Thomas, 1999; Ramsauer et al., 2002). These components, together with the neurons, form a neurovascular unit (Hawkins and Davis, 2005; Nishoku et al., 2008). Pericytes seem to play an important back-up role in the selectivity of the blood-brain barrier (Broadwell and Salcman, 1981; Farrel et al., 1987), limiting the passage of macromolecules (Cancilla et al., 1972; van Deurs, 1976; Broadwell and Salcman, 1981; Brierley and Brown, 1982; Sumner, 1982; Heinsen and Heinsen, 1983; Bär and Budi-Santoso, 1984; Jeynes, 1985). Indeed, in experimental and pathological conditions in which the blood-brain barrier becomes leaky, pericytes are involved in the phagocytosis of foreign proteins that pass the endothelial layer, increasing the number of their lysosomes (Torack, 1961; Baker et al., 1971; Cancilla et al., 1972; van Deurs, 1976; Sumner, 1982). Pericytes augment the blood-brain barrier integrity in EC cultures (Nakagawa et al., 2007) and appear to act as a key factor in modulating functions of neurovascular units (Nishoku et al., 2008). The role of pericytes in the induction and up-regulation of blood-brain barrier functions is mediated by TGF-β production (Dohgu et al., 2005; Takata et al., 2007). Recently, pericyte detachment from the basement membrane and microglial activation have been observed in an induced model of septic encephalopathy, suggesting that these findings may be involved in the mediation of blood-brain barrier disruption in brain inflammatory processeses (Nishoku et al., 2008).

Other Peculiar Pericyte Locations occur, such as pericytes of cochlear stria vascularis, which adopt a peculiar disposition and appear as elongated cells that connect separate capillaries. Therefore, more bridging is observed in this location than in other tissues (Ando et al., 1999).

Pericyte changes associated with aging

Pericytes in aged rat brain cortex increase in number, show hypertrophy, and their processes contain inclusions of different textures (Peinado et al., 1998). Nevertheless, a decrease in the number of pericytes with increasing age in human cerebral white matter, but not grey matter, has been described (Stewart et al., 1987). The basement membrane surrounding the microvessels increases in thickness with age and the range of microvessel wall thicknesses augments linearly according to the amount of pericyte covering (Baker et al., 1971).

Pericytes in pathology

Pericytes are directly involved in several processes (Yamagishi et al., 1999; Allt and Lawrenson, 2001), such as repair through granulation tissue, tumors (pericyte-derived tumors, and pericytes in cancer angiogenesis and metastasis), diabetic microangiopathy (Nakamura et al., 1995; Imesch et al., 1997, McLennan et al., 1999, Koya et al., 2000), fibrosis, atherosclerosis, hypoxia, hypertension (Wallow et al., 1993) and calcific vasculopathies (Yamagishi et al., 1999), as well as other processes including, myosclerosis myopathy, chronic venous insufficiency, Kaposi sarcoma, pulmonary hypertension, Alzheimer’s disease, multiple sclerosis, HIV leukoencephalopathy, trauma (Dore-Duffy et al., 2000), Reynaud’s syndrome and CNS dementias (Verbeek et al., 1997, 1999).

Pericytes in repair trough granulation tissue

In a recent work, we considered the role of pericytes as important mesenchymal precursor cells during repair to granulation tissue (Díaz-Flores et al., 2009), which involves coagulation, inflammation, angiogenesis,
Pericytes and mesenchymal cell niche
proliferation of mesenchymal cells, vascular involution and remodelling (see Díaz-Flores et al., 2009).

Tumors derived from pericytes

Since pericytes are considered to be progenitor cells with great mesenchymal potential (see Pericyte function as progenitor cell. Pericyte plasticity), they may contribute to several soft tissue and bone tumors. For example, transitional findings between pericytes and neoplastic cells are present in myxoid liposarcoma (Fig. 10A), and neoplastic cells in a pericytic location, contacting with endothelial cells, like pericytes, are observed in osteoblastoma (Fig. 10B). Therefore, from a broad perspective, the concept of pericytes as possible progenitors of several soft tissue and bone tumors is a topic that needs future consideration.

According to a current and restricted concept (Fletcher et al., 2002), only some forms of haemangiopericytoma (e.g. sinonasal haemangiopericytoma) and lesions showing evidence of differentiation towards myoid/contractile perivascular cells, such as myopericytoma, glomangiopericytoma, myofibromatosis, myofibroma and infantile haemangiopericytoma, which probably form a morphological continuum, are related to pericytes (perivascular cells). Likewise, some cells of the angioleiomyoma share findings with pericytes (Fig. 10C).

Traditionally, the principal tumor linked with pericytes was the haemangiopericytoma. Indeed, in 1942; Stout and Murray postulated a pericytic origin for a type of tumor, which they named haemangiopericytoma. Since then, this histogenesis has been maintained by several authors. Currently, conceptual changes have included most cases of haemangiopericytoma as solitary fibrous tumors, suggesting a fibroblastic/myofibroblastic origin. Therefore, haemangiopericytomas and solitary fibrous tumors are closely related, if not synonymous (Guillou et al., 2002). Since pericytes do not have a specific marker, it is not surprising that this problem is also inherent to their tumoral counterpart. Furthermore, the existence of the haemangiopericytoma entity has even been questioned (Fletcher, 1994). Haemangiopericytomas and solitary fibrous tumors are mesenchymal neoplasms of adult life and ubiquitous location. They tend to be well-circumscribed and show a thin-walled branching vascular pattern, frequently exhibiting a staghorn or antler-like configuration. Around this elaborate vasculature, closely-packed round to fusiform (Fig. 10D) or, less frequently, spindle cells are observed, presenting small nuclei, eosiophilic or pale cytoplasm and indistinct borders. The ultrastructure is variable, and undifferentiated cells (Erlandson and Woodruff, 1998) or cells with features comparable to those of pericytes or transitional forms with SMC (Rosai, 1969; Battifora, 1973; Nunnery et al., 1981; Kuhn and Llombart-Bosch et al., 1982) have been described. The tumoral cell immunophenotype demonstrates positivity for CD34 (90% to 95% of cases) (Fig. 10E) (Suster et al., 1995; Mentzel et al., 1997; Nielsen et al., 1997; Brunnemann et al., 1999; Hasegawa et al., 1999; Vallat-Decouvelaere et al., 1998; Westra et al., 2000; Guillou et al., 2002), vimentin, CD99 (70% of cases) (Renshaw, 1995; Guillou et al., 2002) and a lower percentage for bcl2 (Chilosi et al., 1997; Suster et al., 1998) and SM actin. CD31 is negative. Type IV collagen and laminin are expressed between ECs and tumoral cells, and sometimes between the latter. Myxoid changes similar to those of myxoid liposarcoma may occur in the interstitium. Break point in 12q13-15 and 19q13 are the most frequent chromosomal aberrations and, among genomic imbalances, loss of segment in 3p, 12q, 13q, 17p, 17q, 19 and entire chromosome 10, and gain of 5q sequences are the most recurrent (Guillou et al., 2002). Insulin-like growth factors and insulin-like growth factor receptors can be expressed in tumor cells (Höög et al., 1997; Paveli et al., 1999), and large tumors may present with hypoglycemia (Simon and Greene 1964; Paullada et al., 1968; Baker et al., 1992; Bainbridge et al., 1997; Paveli et al., 1999; Rose et al., 1999). Peculiar related forms include lipomatous haemangiopericytomas, which contain adipocytes in variable amounts (Taccagni et al., 1993; Mentzel et al., 1997; Vallat-Decouvelaere et al., 1998; Ceballos et al., 1999; Folpe et al., 1999). Meningeal haemangiopericytomas, classically regarded as angioblastic meningiomas, are currently considered to be non meningotelial, since their ultrastructure and immunophenotype are similar to haemangiopericytoma (Nakamura et al., 1987; Guthrie et al., 1989; Winek et al., 1989; D’Amore et al., 1990; Mena et al., 1991; Nemes, 1992; Kleihues et al., 1993).

Pericytes have been taken into account as progenitor cells of some tumors of uncertain differentiation and not included among soft tissue and bone tumors. For example, the neoplastic stromal cells (Fig. 10F) of the CNS capillary hemangioblastoma have been considered...
closely related to pericytes and SMCs (Kamitani et al., 1987).

**Pericytes in tumor angiogenesis**

Constant formation, regression and remodelling of poorly organized vasculature occur in tumors, with abnormal EC and pericyte behaviour (Folkman, 2000; Morikawa et al., 2002; Bergers and Benjamin, 2003). The tumor vessels are immature, tortuous, dilated and hyperpermeable (Morikawa et al., 2002; Baluk et al., 2005; Jain, 2005; Song et al., 2005; Hall, 2006), and the chaotic network of proliferating vessels loses control and cannot terminate vessel growth (Berger et al., 2005). Modifications in the regulatory signaling and in the extracellular matrix in tumors are involved in these changes (Gerhardt and Semb, 2008), and pericyte deficiencies could be partly responsible for vessel abnormalities (Gerhardt and Semb, 2008). Pericyte incidence and modification in tumors (Abramsson et al., 2002; Morikawa et al., 2002; Baluk et al., 2005) include: 1) important participation in certain tumors not grouped as pericytic tumors, as occurs in Pyogenic granuloma (Botryomycoma) (Fig. 11A) and in nodular fasciitis (Fig. 11B). 2) Variable recruitment and coverage (Schlingemann et al., 1991; Benjamin et al., 1999; Zaggag et al., 1999; Abramsson et al., 2002; Morikawa et al., 2002; Baluk et al., 2005; Chantrain et al., 2006) (several studies demonstrating different pericyte coverage of blood vessels of human and animal tumors, such as renal, prostate, mammary and colon carcinoma, glioblastoma multiform, pancreatic islet cell tumors, T241 fibrosarcoma and KRTB osteosarcoma (Wesseling et al., 1995; Eberhard et al., 2000; Abramsson et al., 2002; Morikawa et al., 2002; Baluk et al., 2005; Jodele et al., 2005). Variable coverage may even occur in the same tumor. For example, in Kaposi sarcoma, in which, depending on its histological stage, EC tubes with pericytes (Fig. 11C) but scant basement membrane, and EC tubes surrounded by basement membrane but lacking in pericytes (Fig. 11D) are gradually observed. Finally, when the histological stage progresses towards spindle cells, fragmentation and disappearance of the pericyte sheath occurs (Dictor et al., 1991). 3) Loss of pericyte-EC association with imbalanced interactions. 4) Aberrant cell shape with extension of pericyte processes into the tumor parenchyma. 5) Altered expression of marker proteins (e.g. expression of αSMA actin in capillary). And 6) Modifications of basement membrane, which appears multilayered (Fig. 11E), with collagen of long space pattern (Fig. 11F and insert) and/or loosely associated (Morikawa et al., 2002). Pericyte abnormalities, especially the partial dissociation of pericytes (Hobbs et al., 1998; Hashizume et al., 2000), contribute to increased vascular permeability (accessibility of drugs Hashizume et al., 2000), which alternate with poor perfusion and extensive tumoral cell hypoxia in other tumoral regions. Moreover, the vessels tend to be more haemorragic, due to vascular fragility, and express novel molecules (St. Croix et al., 2000).

**Pericytes in tumoral cell metastasis**

Tumor growth can be accompanied by local invasion in which the cadherin family participates. Invasiveness in malignant neoplasms enables them to reach the routes for metastasis: lymphatics, blood vessels, body surfaces and cavities. Nevertheless, invasion and metastasis are independent pathological traits (Nguyen and Massagué, 2007). Choice of route depends on tumor location and on vascular and tumor characteristics (Wong and Hynes, 2006; Gerhardt and Semb, 2008).

Lymphatic metastasis is facilitated by the absence of pericytes in normal lymph vessels. In addition, the prominent development of lymphatic capillaries in the periphery of many tumors ( Muller et al., 2001; Skobe et al., 2001; Alitalo and Carmeliet, 2002; LeBedis et al., 2002; Schoppmann et al., 2002; Nisato et al., 2003; Streit et al., 2003; Alitalo et al., 2005; Shayan et al., 2006; Gerhardt and Semb, 2008), the presence of wide EC gaps, the intraluminal negative pressure (fluid flux towards lymph vessels) and the active attraction of tumor cells by associated macrophages in lymph vessels and lymph nodes participate in this choice of route (Muller et al., 2001; LeBedis et al., 2002; Schoppmann et al., 2002).

Blood metastasis is limited by pericytes (Xian et al., 2006), since improper pericyte integration into the microvessel wall with deficient coverage, attachment and function is sufficient to trigger tumor cell spreading (Gerhardt and Semb, 2008). Since PDGF-B acts in pericyte migration recruitment and attachment, and sulphation degree of proteoglycans (HSPG) controls PDGF-B retention in altered tumor vessels (Gerhardt and Semb, 2008), matrix metalloproteinases, among other actions in tumor metastasis, may intervene, via actuation on retention of PDGF-B, as a regulator of pericyte recruitment (Chantrain et al., 2006). Xian et al., (2006) have pointed out that neural cell adhesion molecule (NCAN) limits tumor cell metastasis and that this role is mediated by an effect on tumor vessel pericyte recruitment and on perivascular deposition of matrix extracellular molecules. Therefore, NCAN deficiency during tumor progression results in detachment and dysfunction of pericytes with deficient pericyte/EC interactions (Xian et al., 2006). The latter are probably related to extracellular matrix molecules responsible for PDGF-B retention and for Angiotensin 2 expression (Gerhardt and Semb, 2008).

On the other hand, after vascular invasion, the potential extravasation ability of circulating tumor cells may also be dependent on changes in blood vessel stability (Lindblom et al., 2003), in which the pericytes of the regions secondarily involved appear modified. For example, the number of hepatic stellate cells increases in liver metastasis (Enzan et al., 1994) and their activation is tumor dependent. These cells also participate in the progression of metastatic liver cancer (Olaso et al.,
Fig. 11. Pericytes in tumor angiogenesis. A, B. Numerous pericytes (P) and transitional cell forms between pericytes and myofibroblasts (TC) in Piogenic granuloma (A) and Nodular fascitis (B). EC: Endothelial cells. L: Vessel lumen. C, D. Variability of pericyte coverage in Kaposi sarcoma. E, F. Basement membrane around pericytes and ECs appears multilayered in a thyroid medullary carcinoma (E) and with collagen of long space in a neuroblastoma (F -arrow- and insert). Ultrathin sections. Uranyl acetate and lead citrate. x 15000; insert, x 22000
Pericytes and diabetic microangiopathy

Pericyte loss can also occur in capillary beds of humans with diabetes (Tilton et al., 1981), with considerable expression in the retina, wherein the early changes include degeneration and loss of retinal capillary pericytes (pericyte dropout is an important finding in diabetic retinopathy), selective disruption of pericyte/endothelial cell relation and microaneurysm formation (Cogan et al., 1961; Kuwabara and Cogan, 1963; De Oliveira, 1966; Cogan and Kubawara, 1967; Speiser et al., 1968; Ashton and Tripathi, 1977; Sima et al., 1985; Robinson et al., 1989; Tilton, 1991; Hirsh and D’Amore, 1996; Hammes et al., 2002). Subsequently, a progressive and non-proliferative stage of diabetic retinopathy develops, with vascular occlusion (acellular-occluded capillaries), thickening of the vascular basement membrane, increase of vascular permeability and haemorrhages of retinal blood vessels (Benjamin et al., 1998). Finally, a proliferative stage may develop, with angiogenesis in the retina and possible proliferation in the vitreous (Aiello et al., 1998). Proliferative diabetic retinopathy develops when pericyte density is less than 50% (Enge et al., 2002). A number of causes for diabetic pericyte dropout have been pointed out (Motiejunaite and Kazlaukas, 2008), such as: a) decline in PDGF-ß levels (diabetic mice have a reduced expression of PDGF-ß - Tani et al., 2006; and PDGF-ß ablation mimics diabetic retinopathy - Enge et al., 2002); b) up-regulation of angiopoietin 2 (Hammes et al., 2004); c) accumulation of toxic components (advanced glycation end products and polyols as a result of hyperglycemic stress) with increased production of reactive oxygen species and pericyte apoptosis; the via followed by some of these components may be a reduction of PDGF signaling and an increase in the ratio angiopoietin 2/angiopoietin 1 (Hohman et al., 1989; Yamagishi et al., 1995; Stitt et al., 1997; Yamagishi and Imaizumi, 2005; Tani et al., 2006); d) modification of endothelin 1 action in pericytes by elevated glucose levels (De la Rubia et al., 1992), and e) antipericyte circulating antibodies (Attawia and Najak, 1999). Genetic changes in Sorcs 1 (sorting receptor related consensus sequence 1), a gene associated with onset of type II diabetes that binds PDGF, may promote β-cell failure by affecting pericytes in the pancreatic islet capillary network (Clee et al., 2006; Hermey et al., 2006).

The role of the pericyte glycosaminoglycan metabolism in the thickening and increased porosity of the microvascular basement membrane of diabetics has been pointed out (Stramm et al., 1987). Recently, insulin treatment has been shown to prevent H2O2-induced NF-B and caspase-8 activation, as well as apoptosis via IRS1/PI3K/Akt2/FOXO-1 in pericytes, suggesting that this may explain how insulin delays the progression of microvascular complications induced by diabetes (Gerald et al., 2008).

Pericytes and fibrosis

The origin of myofibroblasts is a key to understanding fibrotic processes, such as hypertrophic scars and keloids, idiopathic interstitial pulmonary fibrosis and airway remodelling in asthma, obstructive fibrosis of the kidney and nephrogenic systemic fibrosis (nephrogenic fibrosing dermopathy), reactive fibrosis (chronic pancreatitis and cystitis), systemic sclerosis and tumour stroma fibrosis. Multiple origins have been hypothesised for myofibroblasts (Hinz et al., 2007), the principal sources postulated being preexisting fibroblasts, bone marrow-derived circulating mesenchymal progenitors (bone marrow stromal cells and fibrocytes) and pericytes (Díaz-Flores et al., 2009). Our observations and those of other authors described above suggest that pericytes, detached from the vessel walls, migrate, acquire a fibroblast-like phenotype and act as collagen-producing cells. Examples of pericyte participation in fibrotic processes are liver persistent injury, in which hepatic stellate cells are involved in fibrosis, cirrhosis and portal hypertension (Reynaert et al., 2002), and obstructive fibrosis of the kidney, in which collagen producing pericytes have been identified as the major source of interstitial myofibroblasts (Lin et al., 2008). Likewise, sequential microvascular and perivascular changes have been described during fibrosis (Prescott et al., 1992) as occurs in dermal scarring (Sundberg et al., 1993, 1996), tumor stroma fibrosis (Sundberg et al., 1996), systemic sclerosis and in autoimmune Raynaud’s phenomenon (Rajkumar et al., 1999).

Pericytes in atherosclerosis and calcific vasculopathies

Neovascularization in atherosclerotic plaques is a well-known finding, which contributes to the progression of atherosclerosis (intraplaque contribution of nutrients, haemorrhage and cytokine secretion). 3G5-positive pericytes have been observed within the media and around some microvessels in human atherosclerotic vessels, with expression of hepatocyte growth factor (HGF) and its receptor (a transmembrane tyrosine kinase encoded by the proto-oncogene c-Met), which may be involved in neovascularization and in pericyte recruitment (by potent chemotactic effects of HGF on pericyte migration), mediated via the PI3K pathway and AKT phosphorylation (Liu et al., 2007). Furthermore, neovascularization may provide pericytes, as a source of progenitor cells. Indeed, the possible origin of myointimal cells in atherosclerotic lesions is as follows: SMCs and SMC related cells of the arterial media layer, adventitial progenitor cells and bone-marrow-derived circulating cells (Díaz-Flores et al., 2009). Fibroblasts and vasa-vasorum pericytes are the principal candidates
in the adventitial hypothesis on the origin of the arterial myointimal cells (Díaz-Flores and Domínguez, 1985; Hu et al., 2004; Torsney et al., 2005; Stenmark et al., 2006). In this way, microvessel penetration into the arterial wall from the adventitial layer may not only occur in the later stages of atherosclerosis, but also in the earliest stages of its precursor lesions, such as some forms of intimal thickening (Díaz-Flores and Domínguez, 1985; Madrid et al., 1998), in which a transitory and brief neovascularization with pericyte proliferation in the intimal region is followed by a rapid microvascular involution, suggesting that pericytes contribute myointimal cells (hypothesis of pericytes as source of myointimal cells - Díaz-Flores and Domínguez, 1985). In this context, it has been postulated that arterial intimal thickening results from a similar mechanism to that of the organization of thrombus, with subsequent events depending on whether or not the arterial circulation has been interrupted. On interruption, there is both a penetration of the vasa-vasorum and myointimal differentiation from pericytes, perivascular fibroblasts or circulating progenitors cells, whereas when the arterial circulation has remained unchanged there is no vasa-vasorum penetration and the intimal thickening originates from pericytes and perivascular fibroblasts migrating from the arterial vasa-vasorum and adventitia, pericyte-like cells from the arterial media and intima layers and from circulating progenitor cells (Díaz-Flores and Domínguez, 1985, Díaz-Flores et al., 2007, 2009). Thus, using a technique that marked vasa-vasorum pericytes, it was demonstrated that a population of myointimal cells (myofibroblast-like) originates from the labeled pericytes during arterial intimal thickening formation, (Díaz-Flores et al., 2007). In experimental atherosclerosis induced in the rat, we have demonstrated that most pericytes show abundant lipidic vacuoles (Fig. 12A,B). The aberrant differentiation of pericytes may contribute to the development of calcific vasculopathies (Kirton et al., 2007).

**Pericyte modifications in other pathological processes**

Increased numbers of pericytes have been described in autosomal recessive myosclerosis myopathy (Merlini et al., 2008).

Abnormal presence of pericytes in lymph vessels plays an important role in human lymphedema distichiasis. In this process, lymphatic ECs present lower expression of Foxc2 (mutation in FOXC2 gene), required for the establishment of a pericyte-free lymphatic capillary network (see pericytes and lymphatic vessels). The absence of Foxc2 changes lymphatic EC phenotype, increasing PDGF-β expression and subsequently recruitment of pericytes with narrowing of the lymphatics and lymph stasis (Petrova et al., 2004).

Changes in pericyte coverage occur in the skin microvasculature of patients with chronic venous insufficiency (Laaff et al., 1991). Thus, the destruction of the pericyte envelope accompanies typical glomerulum like alterations of cutaneous capillaries and might lead to microcirculation dysfunction and leg ulcers in chronic venous insufficiency (Laaff et al., 1991).

Deficit of pericyte recruitment (pericyte deficiency) appears to be a cause for pulmonary hypertension in Adams-Oliver syndrome (Patel et al., 2004).

In the central nervous system, pericyte involvement has been described in several processes, such as Alzheimer’s disease, autoimmune diseases and infectious encephalitis (Thomas, 1999). Thus, pericytes are among the cells forming amyloid in the vessel wall in Alzheimer’s disease (Wegiel and Wisniewski, 1992; Wisniewski et al., 1992). Due to their capacity as macrophages, pericytes intervene in immunological properties of the Virchow-Robin space (Esiri and Gay, 1990), as occurs in multiple sclerosis and in human immunodeficiency virus (HIV) leukoencephalopathy (Smith et al., 1990).

**Clinical and therapeutic implications of pericytes**

At present, action on pericytes may be undertaken by interfering in or enhancing EC/pericyte communication (Jain and Booth, 2003b).

**Desestabilization of vessels (interference of EC/pericyte communication)**

Since pericytes, like EC, undergo molecular changes during neovascularization, they may be targeted for antiangiogenic therapy (Berger et al., 2005). Indeed, combinations of antipericyte agents with antiendothelial agents in antiangiogenic therapies may be used with synergistic effects (Bergers et al., 2003), which provide more benefits than by simply targeting ECs alone (Bergers et al., 2003; Erber et al., 2004). Indeed, the combined targeting of pericytes and ECs in antiangiogenic therapies may act to prevent neovascularization, as well as to reduce the established vessels in the tumors and other angiogenesis-dependent diseases. Consequently, by means of this combined targeting, the resistance of treatment conferred by pericytes is avoided, enforcing tumor vessel regression by interfering with pericycle-mediated EC survival mechanisms (Wang et al., 2007), including EC apoptosis, blood vessel regression and tissue hypoxia (Dore-Duffy et al., 2006). Examples of these combined therapies are the blockade of VEGFR-2 and PDGR-β signalling with kinase inhibitors (Shaheen et al., 2001; Bergers et al., 2003; Dore-Duffy et al., 2006) or the association of PDGF pathway inhibitors with antiangiogenic chemotherapy that targets ECs (Pietras and Hanahan, 2005). These combinations enable the regression of late-stage tumors (Bergers et al., 2003; Erber et al., 2004). Pathological angiogenesis may also
be reduced by targeting other pericyte markers, such as NG2 proteoglycan (Minasi et al., 2002; Maciag et al., 2008). Indeed, using a Lysteria-monocyte gene-based vaccine against high molecular weight melanoma-associated antigen protein (HMW-MAA or NG2), the pericytes in the tumor vasculature are targeted, which might contribute to the improved efficacy of this vaccine in slowing down tumor growth (Maciag et al., 2008).

**Formation of a stable vasculature (enhancement of EC/pericyte communication)**

Therapeutic increase of pericyte recruitment to form a stable microvasculature is an objective in diseases that show degeneration and loss of vessel pericytes, such as diabetic retinopathy. In this way, control of pericyte/EC interactions is one of the aims of tissue engineering, in which the procedures to develop multi-tissue organs, combining scaffolds, cells and/or biomolecules, require the optimization of conditions for the induction of cell adhesion, migration, proliferation and differentiation, as well as angiogenesis and production of new extracellular matrix.

**Therapeutic promise**

The use of pericytes or subsets of pericytes as progenitor cells, with great mesenchymal potential and as a source of undifferentiated cells, is a therapeutic promise, for example, to engineer different tissues both “ex vivo” and “in vivo”. This possibility will increase when the requirements for favouring cell renewal over differentiation during expansion in culture is better understood. Likewise, intratumorally grafted pericyte-like MSCs might be a vector system for delivering molecules to affect tumor angiogenesis and for targeting

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**Fig. 12. Pericytes in experimental atherosclerosis.** Abundant lipidic vacuoles (arrows) are observed in pericytes (P). EC: Endothelial cell. L: Vessel lumen. A: Semithin section. Toluidine Blue. B: Ultrathin section. Uranyl acetate and lead citrate. A, x 250; B, x 13000
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Pericytes and mesenchymal cell niche


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