

Microvascular Pericyte Contractility In Vitro: Comparison with Other Cells of the Vascular Wall

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Abstract. Collagen lattices containing bovine retinal pericytes (RPs), vascular smooth muscle cells (VSMCs), pulmonary microvessel endothelial cells (PMECs), or aortic endothelial cells (AECs) were prepared and contraction was quantitated by measuring the resulting change in lattice area. VSMCs were the most efficient at lattice contraction followed by RPs and then PMECs. AECs did not contract the lattices. To document further that these observations represent contraction, cells were grown on inert silicone rubber sheets. Substratum wrinkling was indicative of tension development and quantitated as percent of cells contracted. RPs were more contractile than PMECs, and AECs were incapable of developing tension. VSMCs were less contractile than RPs, unlike the comparative contractility observed with the lattice system. Altera-

tion of actin-containing filaments by cytochalasin B significantly reduced RP contraction of silicone rubber and inhibited their contraction of collagen lattices in a dose-dependent manner. Rhodamine-phalloidin staining of contracting RPs revealed microfilament bundle orientations that suggested their association in the force applied for contraction. RP, VSMC and PMEC contraction of collagen lattices was directly proportional to the concentration of fetal calf serum. Also, RP contraction was greater in calf serum than calf plasma-derived serum, an indication that RPs respond to substances that appear continuously and episodically in blood. These *in vitro* findings support the theory that pericytes *in vivo* are contractile but that endothelial cells may also contribute to microvascular tonus.

MICROVASCULAR pericytes are intramural cells that envelop the endothelial cell (EC)¹ lining of all blood vessels from arterioles to postcapillary venules (18). Pericytes throughout the capillary network are morphologically diverse but generally appear as extensively branched cells with longitudinal and circumferential processes (32). Their diverse morphology is undoubtedly matched by functional diversity.

It has been often postulated that pericytes are contractile cells and contribute to the regulation of blood flow at the microvascular level. Ultrastructural investigations have described transitional cell forms between pericytes and smooth muscle cells (19, 32), and have localized abundant actin microfilaments within pericytes (17, 30). Both smooth muscle and nonmuscle isoactins were identified as constituents of these filaments *in situ* and *in cell culture* by immunofluorescence procedures (11). Two additional proteins essential for contraction, myosin and tropomyosin, were immunocytochemically identified in pericytes and found to be present in significantly greater amounts than in other nonmuscle cells

(14, 15). Cyclic GMP-dependent protein kinase, a protein postulated in the regulation of smooth muscle contraction, was also found in high amounts in pericytes (13). Taken together, these morphological and biochemical data suggest that pericytes are cells equipped for a contractile function.

Indirect evidence of pericyte contractility was provided by Tilton et al. (27). In response to perfusion of skeletal muscle capillaries with vasoactive agents, selective buckling of ECs beneath pericytes and reduced capillary diameters were observed ultrastructurally. However, these studies could not definitively distinguish between endothelial shape change due to pericyte contraction and contraction by the ECs themselves. To investigate the contraction of each of these cell types separately and estimate the relative contributions of each cell type to the contractility of the capillaries, we examined the contractile abilities of vascular cells *in vitro*. To obtain these data, we used modifications of two *in vitro* systems developed previously to assess the contractility of cultured cells (1, 7, 10). The contraction of collagen lattices and, separately, silicone rubber substrata by large and small vessel cells were compared. Our observations indicate that bovine retinal pericytes (RPs) produce a contractile response that is different than that of bovine pulmonary microvessel endothelial cells (PMECs), aortic endothelial cells (AECs), and vascular smooth muscle cells (VSMCs) *in vitro*.

1. *Abbreviations used in this paper:* AEC, aortic endothelial cell; DiI-Ac-LDL, acetylated low density lipoprotein labeled with 1,1'-dioctadecyl-1-3,3,3',3'-tetramethyl-indo-carbocyanine perchlorate; EC, endothelial cell; PDS, plasma-derived serum; PMEC, pulmonary microvessel endothelial cell; RP, retinal pericyte; VSMC, vascular smooth muscle cell.

Materials and Methods

Cell Culture

RPs were derived from isolated bovine retinal microvessels as described by Gitlin and D'Amore (8). Briefly, excised retinas were finely minced with scalpel blades and digested with collagenase. The tissue digest was filtered through a nylon mesh and the filtrate was centrifuged (100 g for 3 min) and resuspended in DME (Gibco, Grand Island, New York) supplemented with 5% FCS (HyClone Laboratories, Sterile Systems, Inc., Logan, UT). The capillary fragments were seeded into tissue culture flasks and maintained at 37°C in a humidified atmosphere. The tissue culture plastic substratum promoted the growth of RPs but not retinal microvessel ECs. RPs were distinguished from ECs based on morphology, growth pattern, and the absence of staining with acetylated low density lipoprotein labeled with 1,1'-dioctadecyl-1-3,3',3'-tetramethyl-indo-carbocyanine perchlorate (DiI-Ac-LDL; Biomedical Technologies, Inc., Cambridge, MA) (29). RPs were distinguished from smooth muscle cells by their slower growth rate and lack of "hill and valley" appearance at confluence. First passage RPs were used in all experiments.

VSMCs were obtained from medial explant outgrowths of bovine aortas according to the method of Ross (22) and grown in DME supplemented with 10% FCS. All experiments were performed with first passage VSMCs.

PMECs were derived from peripheral sections of bovine lungs as described previously (3) and cultured in DME supplemented with 5% horse plasma-derived serum (PDS) (HyClone Laboratories, Sterile Systems, Inc.), 20 µg/ml endothelial cell growth factor (Collaborative Research, Inc., Lexington, MA), and 90 µg/ml heparin (Sigma Chemical Co., St. Louis, MO). Confluent cultures were identified as endothelial based on morphological and immunological criteria and uptake of fluorescent DiI-Ac-LDL. Cells from subculture Nos. 7-10 were used.

AECs were isolated from bovine aortas as described previously (25) and cultured in DME supplemented with 10% FCS. Cultures were identified as endothelial based on morphological and immunological criteria, including DiI-Ac-LDL uptake. All experiments were performed using first passage AECs.

Matrix Preparations

Three-dimensional collagen lattices were prepared by a modification of the method of Bellows et al. (2). Concentrated (10×) α-MEM (0.5 ml) (Gibco), 0.26 M sodium bicarbonate (0.5 ml), 10× concentrated antibiotic-antimycotic (0.5 ml) (Sigma Chemical Co.), Vitrogen 100 (1.8 ml) (3.3 mg/ml bovine dermal collagen in 0.012 N HCl; Collagen Corp., Palo Alto, CA), and 1 N NaOH (0.07 ml) were mixed together in chilled plastic tubes. Cultured cells harvested with 0.05% trypsin-0.02% EDTA (Gibco) were centrifuged at 100 g for 4 min and resuspended in α-MEM at the required cell density. The appropriate concentrations of FCS and cells were added to the collagen mixtures. The same solutions were prepared without cells for use as controls. The final collagen concentration was 1.2 mg/ml. Aliquots (0.5 ml) of the mixtures were pipetted into each well of a 24-well tissue culture dish. The dishes were placed in a 37°C humidified incubator where polymerization of the collagen took place within 10 min. At that time the lattices were detached from the dishes to allow uniform contraction and their diameters measured at 24 h intervals.

To minimize any differences in cell numbers resulting from different proliferation rates of the cells in collagen lattices, the culture media was not replenished during the experiment and contraction was measured over a 3-d period. Under these conditions, none of the cell types used demonstrated any significant increases in cell numbers.

In experiments to compare the effects of plasma and serum on pericyte contraction of collagen lattices, cells were seeded into lattices containing either 5% or 25% calf serum (HyClone Laboratories, Sterile Systems, Inc.) or 5% or 25% calf PDS. PDS was prepared in the following manner. Blood was collected from calves by exsanguination into prechilled plastic containers with anticoagulant citrate dextrose (Fenwal Inc., Ashland, MA). The anticoagulated blood was transferred to prechilled plastic centrifuge tubes and spun at 1,000 g for 15 min at 4°C. The supernatant was re-spun at 5,000 g for 15 min at 4°C. The supernatant from the second spin was collected and spun at 15,000 g for 15 min at 4°C. The resulting supernatant was defibrinated by heating at 57°C for 30 min. The fibrin clot was pelleted at 1,200 g for 15 min and the resulting PDS was filtered through a 0.45-µm filter. The PDS was shown to be devoid of platelet-derived components using a 3T3 cell DNA synthesis assay.

Silicone rubber substrata were prepared by a modification of the meth-

od of Harris et al. (10). A small volume of dimethylpolysiloxane (silicone fluid; Sigma Chemical Co.) of the desired viscosity was applied to 12-mm² round glass coverslips. The coverslips were placed on the caps of 15 ml centrifuge tubes and spun at 100 g for 4 min to obtain an even coating of silicone on the coverslips. The coating was heated for 2 s using a Bunsen burner to induce cross-linking of the surface of the dimethylpolysiloxane and formation of the silicone rubber sheets. Dimethylpolysiloxane of 60,000 centipoise (cp) and 12,500 cp viscosity were obtained as standard grades and used directly. Intermediate viscosity silicone (30,000 cp) was prepared by blending 54% by weight of 60,000 cp silicone with 46% by weight of 12,500 cp silicone. After preparation, the coverslips were placed in 24-well tissue culture dishes and sterilized by UV irradiation. Cells (1×10^6) were plated on the coverslips in DME supplemented with 10% FCS.

Collagen films were prepared by applying a thin layer of aqueous Vitrogen 100 to sterile glass coverslips in 24-well tissue culture dishes. The coverslips were allowed to air dry in a laminar flow hood and then rinsed free of acid with PBS.

Measurement of Contraction

When cells were incorporated into collagen lattices, their cumulative contraction pulled the collagen fibers into a dense arrangement and decreased lattice area. To quantitate this contraction, wells containing lattices were placed on top of a metric ruler against a black background and the major and minor axes of the lattices were measured with the aid of a Wild M3 dissecting microscope (6× magnification). The areas of the lattices were computed from the averaged diameters, and contraction was expressed as percent decrease in precontracted area. The precontracted area of a lattice was defined as the area of the well in which it was formed. Diameters of the lattices were measured at 24-h intervals. To estimate the number of cells within a lattice, lattices were transferred to 15-ml centrifuge tubes containing 0.5 ml of collagenase (4 mg/ml) and incubated for 1 h at 37°C in a shaking water bath. Freed cells were counted with a hemocytometer.

When cells were seeded on the surface of silicone rubber sheets, the tension exerted by the cells compressed the rubber beneath them into easily visible wrinkles. The changes induced in the substratum by the cells were observed and photographed using a Nikon inverted microscope with a 20× phase-contrast objective. To quantitate contraction, 100 random cells were counted and scored as either contracted (exhibiting wrinkles) or relaxed (no wrinkles present). The results were expressed as percent of cells contracted.

Pericyte Contraction and Stress Fibers

We examined the intracellular architecture of RPs contracting a Vitrogen film. Wrinkling of the substratum was observed and photographed using a Zeiss Universal microscope with a 25× Neofluar phase-contrast objective. The actin cytoskeletal elements were examined in the same cells. Actin was labeled with rhodamine-phalloidin (Molecular Probes, Inc., Junction City, OR) in the following manner. Cells on Vitrogen films were washed three times with PBS, fixed in 3.7% formalin at room temperature for 5 min, permeabilized by immersion in acetone at -20°C for 5 min, and then washed with PBS. Cells were incubated with rhodamine-phalloidin (1.65×10^{-7} M) for 20 min followed by brief washing with PBS. Cells were examined and photographed with a Zeiss Universal microscope using a 25× Neofluar objective.

The microfilament disrupting agent, cytochalasin B, was tested for its effect on RP contraction. Cytochalasin B (Sigma Chemical Co.) was dissolved in 100% ethanol at 1 mg/ml and diluted to final concentrations of 0.1, 1.0, and 10.0 µg/ml with DME containing 25% FCS.

RPs (2×10^5) were incorporated into collagen lattices in the presence of 25% FCS, allowed to attach for 24 h, and cytochalasin B was added at the concentrations indicated above. Contraction was quantitated at 24 and at 48 h after the addition of cytochalasin B. RPs (1×10^6) were also seeded on 60,000 cp viscosity silicone rubber substrata in DME supplemented with 25% FCS. 48 h later, when contraction was exhibited by 100% of the cells, cytochalasin B (1 µg/ml) was added. 2 h after drug addition, contraction was quantitated.

Statistical Analysis

One-way analysis of variance and Duncan's multiple range test were used to compare groups within experiments using the collagen lattice assay (26). Significant differences between means obtained with the silicone rubber assay were calculated using a standard significance test for dichotomous variables (26).

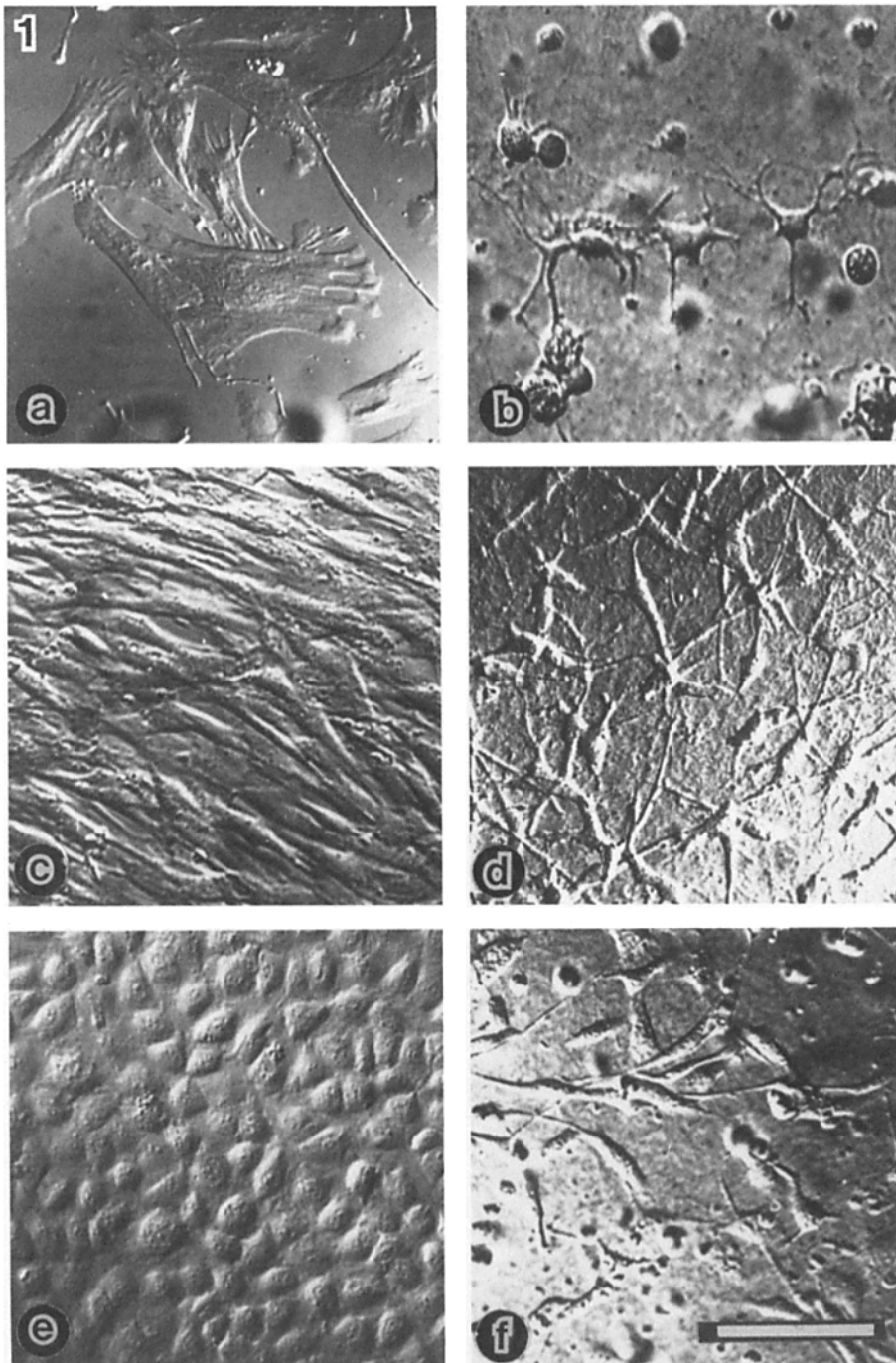


Figure 1. Nomarski micrographs of RPs, VSMCs, and AECs on glass coverslips (*a*, *c*, and *e*, respectively) and within collagen lattices (*b*, *d*, and *f*, respectively). Bar, 100 μm .

Results

Morphology

Nomarski micrographs of RPs, PMECs, AECs, and VSMCs grown on glass coverslips or within collagen lattices depict the different morphologies of the cell types used (Fig. 1). When incorporated into collagen lattices, RPs demonstrated a branched morphology similar to that seen *in vivo*, with cytoplasmic processes extending from a rounded cell body. AECs, PMECs, and VSMCs entrapped in the collagen meshwork demonstrated an elongated morphology with fewer cell

processes. PMEC morphology was identical to that of AECs; thus the micrographs of PMECs are not shown.

Contraction of Collagen Lattices by Vascular Wall Cells

The contraction of three-dimensional hydrated collagen lattices by RPs, VSMCs, PMECs, and AECs was compared and significant cell-specific differences were observed (Fig. 2). Of the vascular cells examined, VSMCs exhibited the most rapid and extensive contraction in the presence of 5% FCS (Fig. 2 *a*), 10% FCS (Fig. 2 *b*), and 25% FCS (Fig. 2 *c*). Maximum contraction was 98% in the presence of 25%

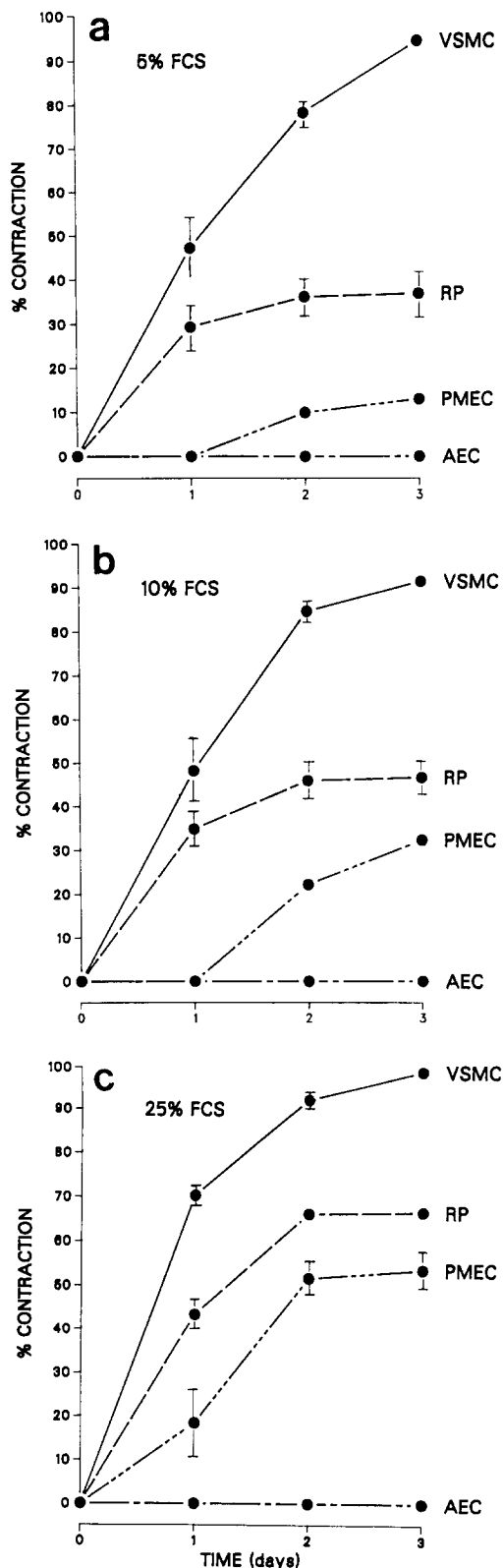


Figure 2. The comparative abilities of vascular wall cells to contract collagen lattices in the presence of varying FCS concentrations. Collagen lattices were prepared with 2×10^5 cells in a final serum concentration of 5, 10, and 25% (a-c, respectively). Each data point represents the average percent contraction of seven lattices \pm SD (when the SD was smaller than the symbol it is not shown). All values between different cell types at a given time point within each concentration of FCS are significantly different from one another at the $P < 0.01$ level of confidence, except day 1 values between

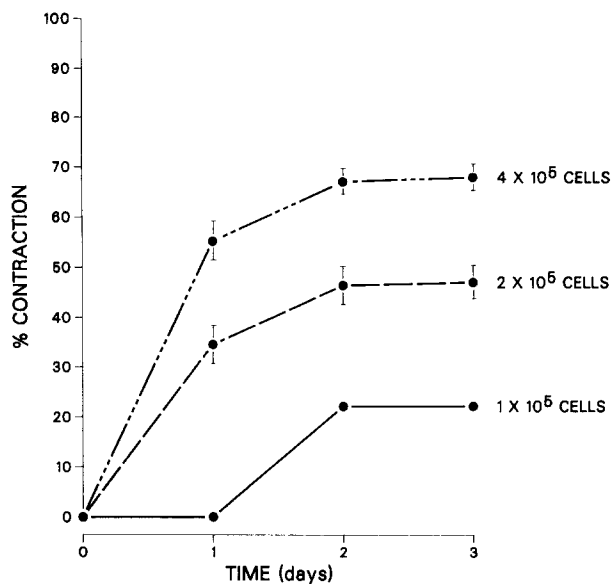


Figure 3. Relationship between contraction of collagen lattices and the number of RPs plated in the lattices. Lattice contraction by 1×10^5 , 2×10^5 , and 4×10^5 RPs in the presence of 10% FCS is shown. Each point represents the average percent contraction of eight lattices \pm SD (when the SD was smaller than the symbol it is not shown). Differences in contraction between lattices containing different numbers of cells on a given day are statistically significant at the 1.0% level of confidence for each measurement shown.

FCS. RP contraction was significantly slower and less extensive than that of VSMCs in all three serum concentrations. RP contraction was maximum at 66% in 25% FCS. PMEC contraction was significantly less than RP contraction in the presence of 5, 10, and 25% FCS. The greatest extent of contraction was observed to be 53% in the presence of 25% FCS. AECs did not demonstrate any contraction; lattices containing AECs and lattices without cells did not undergo any change in area.

The rate of collagen lattice contraction by VSMCs, RPs, and PMECs was directly proportional to the concentration of FCS (Fig. 2). In addition, the extent of lattice contraction by RPs and PMECs was influenced by the FCS concentration. In the presence of 25% FCS, these cells contracted the lattice more rapidly and to a greater extent over a 3-d period than cells maintained in 5 and 10% FCS. Upon termination of the experiment on day 3, lattices were digested and freed cells counted. No significant differences in cell numbers within lattices among the various FCS concentrations used were observed for up to 3 d. This indicated that the serum dependence of collagen lattice contraction by VSMCs, RPs, and PMECs was independent of cell proliferation.

Density Dependence of Pericyte Lattice Contraction

The effect of cell density on the rate and extent of RP lattice

PMECs and AECs in both 5 and 10% FCS. For VSMCs, RPs, and PMECs, differences in contraction between cells in the presence of 5, 10, and 25% FCS are significant ($P < 0.01$) at each time point measured except the following points: for VSMCs, day 1 values between 5 and 10% FCS and day 3 values between 5, 10, and 25% FCS; for PMECs, day 1 values between 5 and 10% FCS.

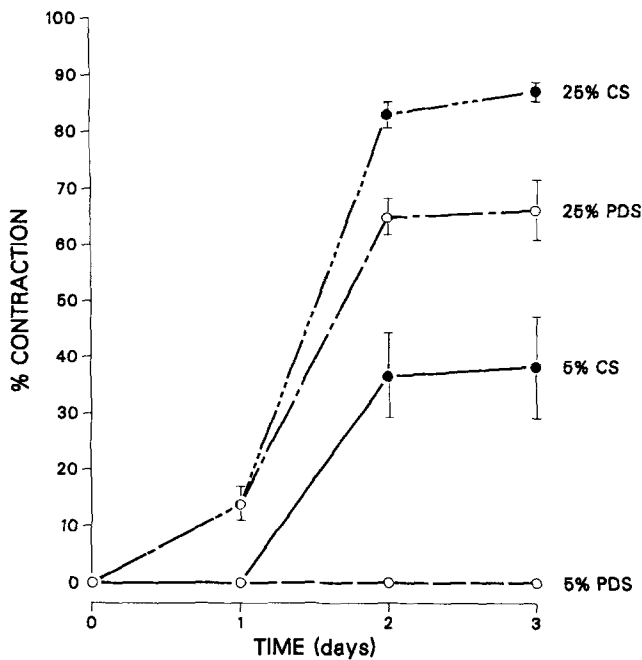


Figure 4. Influence of serum vs. plasma on pericyte contraction of collagen lattices. Lattices were initially seeded with 2×10^5 cells and a final calf serum concentration of 5 or 25%, or a final calf PDS concentration of 5 or 25%. The results shown represent the average \pm SD of eight lattices (when the SD was smaller than the symbol it is not shown). All values between the calf serum (CS) and PDS concentrations represented on a given day are significantly different from one another ($P < 0.01$) except day 1 values between 25% calf serum and 25% PDS and day 1 values between 5% calf serum and 5% PDS.

contraction was demonstrated in a series of experiments that quantified the temporal course of contraction as a function of the number of pericytes present (Fig. 3). The rate and extent of RP lattice contraction were proportional to the initial cell-seeding density. Lattices seeded with 1×10^5 cells did not begin to contract before day 1 as opposed to lattices seeded with 2 and 4×10^5 cells that began contracting during the first 24 h. The contraction of sparsely populated lattices lagged well behind the more densely populated ones throughout the experimental period.

Effect of Serum vs. Plasma on Pericyte Lattice Contraction

The serum dependence of RP lattice contraction was investigated by comparing the effects of calf serum and calf PDS (Fig. 4). Contraction was greater in response to 5% calf serum than 5% PDS and greater in the presence of 25% calf serum than 25% calf PDS over 3 d. No contraction was observed in response to 5% PDS although the cells remained viable as determined by trypan blue exclusion at the end of the experimental period.

Effects of Cytochalasin B on Pericyte Lattice Contraction

The role of actin filaments in the contraction of collagen lattices by RPs was investigated using the microfilament-disrupting agent, cytochalasin B. A dose-response curve of RP contraction in the presence 0.1, 1.0, and 10.0 $\mu\text{g/ml}$ of

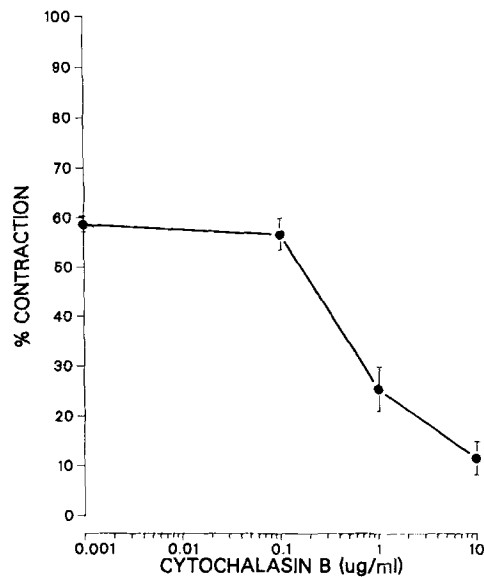


Figure 5. Effect of cytochalasin B on the ability of pericytes to contract collagen lattices. Cells (2×10^5) were incorporated into collagen lattices in the presence of 25% FCS. 24 h after seeding, DME containing 0.0, 0.1, 1.0, or 10.0 $\mu\text{g/ml}$ of cytochalasin B plus 25% FCS was added to each lattice. The same treatments were added again at 48 h. The data shown represent the average % contraction of eight lattices \pm SD as measured at 72 h after seeding.

cytochalasin B is shown in Fig. 5. A significant difference in contraction between nontreated and cytochalasin B-treated cells appeared 24 h after treatment and continued for the remainder of the experiment (72 h after seeding). The degree of contraction was inversely proportional to the concentration of cytochalasin B. Concentrations of 1.0 and 10.0 $\mu\text{g/ml}$ also resulted in arborization of the cells, an effect previously associated with dramatic changes in the microfilament components of cultured cells (24).

Contraction of Silicone Rubber by Vascular Wall Cells

The results described above suggested that when RPs, VSMCs, or PMECs were incorporated into collagen lattices, the cumulative contraction of the cells pulled the collagen fibers together causing a decrease in lattice area. There is a possibility that changes in the dimensions of the lattice by the cells is partially influenced by biochemical alterations of the lattice protein (10) or its dehydration (31). A second assay was therefore used to confirm that substrate change was due to cell contraction and not enzyme action or dehydration.

Cells were grown on the surface of distortable silicone rubber substrata. The tension the cells exerted compressed the surface of this elastically weak material into a series of easily visible wrinkles (Fig. 6). Unlike a collagen substratum, there was no possibility that this inert rubber was being dehydrated or biochemically altered in any way.

The comparative abilities of vascular wall cells to contract silicone rubber substrata of various viscosities (strengths) is shown in Table I. Lowering the viscosity of the rubber results in a weaker, more movable substratum. The results show that RPs generate by far the most tension, with 100% of the cells producing large distortions in the substratum after 48 h of culture on the highest viscosity rubber. The wrinkles generated grew larger and more numerous beneath the cell body

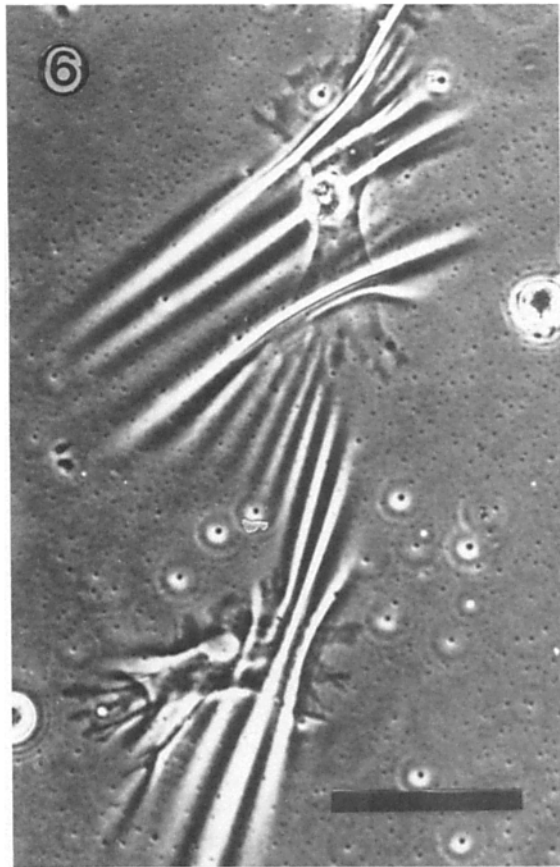


Figure 6. Pericyte tension development on silicone rubber. Pericytes growing on silicone rubber for 24 h were photographed. As can be seen from the phase-contrast micrograph, the tension exerted on the substratum by the cells resulted in the compression of the surface of the rubber into a series of easily visible wrinkles. Bar, 75 μm .

with time. With decreasing viscosity a smaller percentage of RPs exhibited tension. We attributed this finding to difficulties in RP spreading on such a weak substratum.

The ability of VSMCs and PMECs to distort the rubber sheets was far less than that observed for RPs. The highest percentage of cells exhibiting contraction was 29% for VSMCs and 23% for PMECs after 48 h on the lowest viscosity substratum. As the viscosity of the rubber was increased, the ability of these two cell types to contract it decreased. The contraction exhibited by VSMCs on rubber substrata was significantly less than that observed for RPs, an observation different from the results obtained when collagen lattices were used as a substratum.

AECs did not distort even the most flexible (12,000 cp viscosity) substrata. This inability to contract was not due to poor adhesion to the rubber because normal spreading did occur. The results reported demonstrate only individual cell contraction. If the AECs were allowed to grow to confluence on the rubber sheets, the cumulative effort of these many cells produced barely detectable wrinkles.

Role of the Cytoskeleton in Tension Development

RPs cultured on silicone rubber sheets for 48 h were treated with cytochalasin B (data not shown). Alteration of actin-containing filaments by cytochalasin B (1 $\mu\text{g/ml}$; dose deter-

mined by the effect of cytochalasin B on RP contraction of collagen lattices) significantly reduced RP contraction as seen by an 81% decrease ($P < 0.01$) in the percentage of cells wrinkling the substratum compared to controls. This relaxation of tension was visibly evident by a progressive disappearance of the wrinkles in the rubber from 15 min to 2 h after treatment. When cytochalasin B was removed and fresh growth medium added, the effects of cytochalasin B were reversed completely by 24 h.

To implicate cell attachment in the generation of force by RPs, cells growing on silicone rubber sheets for 24 h were treated with trypsin. The rounding up of the cells due to trypsin treatment was associated with a release of tension as indicated by the relief of the wrinkles in, and re-expansion of, the rubber substrata to their original shape. Others have shown that these conditions also result in the rapid disassembly of stress fibers (16).

When pericytes were seeded onto thin sheets of air-dried collagen, they distorted the substratum in a pattern similar to that observed on a silicone rubber sheet (Fig. 7). Because the collagen films were more manipulatable than the rubber, we used them to assess further the role of the cytoskeleton in force generation. Phase-contrast micrographs of pericytes wrinkling collagen films (Fig. 7 *a*), taken together with fluorescent micrographs of these same cells labeled with rhodamine-phalloidin (Fig. 7 *b*) revealed the extra- and intracellular architecture of the cells. Staining of cells with wrinkles beneath revealed large microfilament bundles that arranged primarily at right angles to the wrinkles; an observation in agreement with the theory that stress fibers play a role in generating the force for contraction (12, 28).

Discussion

The wall of nonmuscular microvessels is composed of two cell types, endothelial cells and pericytes. The control of capillary blood flow has been attributed to pericyte contractility (21, 23, 27, 32), endothelial contractility (9), and/or to a combination of the two (21). The lack of consensus in this matter stems partially from the inability to measure separately contractions of either cell in vivo. Therefore, we have used in vitro methods to assess the potential relative contractile contributions of each cell type.

Table I. Contraction of Silicone Rubber by Large and Small Vessel Wall Cells

Cell type	Cells contracted as a function of silicone viscosity		
	12,500 cp	30,000 cp	60,000 cp
	%	%	%
RPs	80	91	100
VSMCs	29	17	1
PMECs	23	14	6
AECs	0	0	0

RPs, VSMCs, PMECs, and AECs were seeded onto films of silicone rubber in the presence of 10% FCS as described in Materials and Methods. The data shown were gathered 48 h after seeding and summarize the results of the percentage of cells contracted on 32 rubber sheets from four separate experiments. Values are expressed as the mean percentage of cells contracted. All values among the different cell types used within a given silicone viscosity were significantly different from one another ($P < 0.01$) except all values between PMECs and VSMCs and values between AECs and both PMECs and VSMCs on 60,000 cp viscosity silicone.

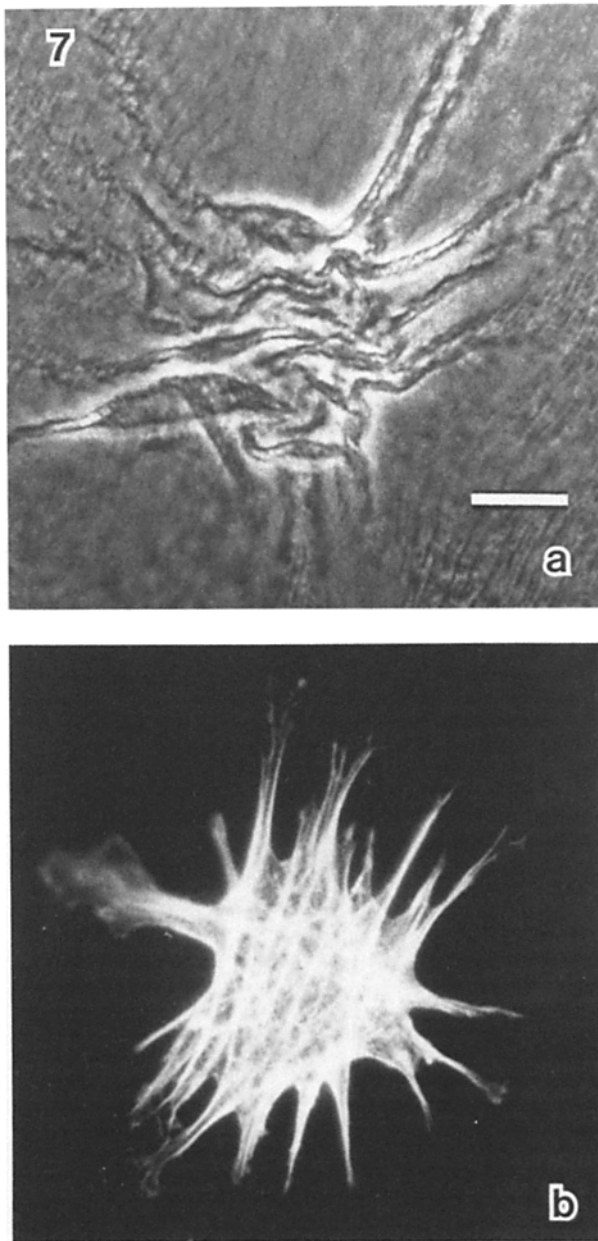


Figure 7. Pericyte tension development on a collagen film and the associated pattern of stress fiber staining. Pericytes seeded onto collagen films distorted the substratum in a manner similar to that observed on silicone rubber. The phase-contrast (a) and rhodamine-phalloidin fluorescent (b) micrographs of the same cell at different focal planes demonstrate that cells showing wrinkles beneath them also show large bundles of F-actin filaments arranged primarily at right angles to the wrinkles, implicating them in the generation of force to contract the collagen. Bar, 20 μ m.

With a collagen lattice as a substrate, the contractile activity of the vascular wall cells was VSMCs > RPs > PMECs; and AECs under identical experimental conditions did not contract. The relative contractilities of these cells *in vitro* are consistent with what might be expected *in vivo*: VSMCs the predominant source of contraction in the aorta, and pericytes the predominant cause of microvessel contraction. However, collagen lattice contraction by PMECs suggests that ECs may also contribute to microvessel contractility.

Contractile differences between cell types on silicone rubber substrata were similar to those observed in the collagen lattice assay with one exception: VSMCs were significantly less contractile than RPs. There is a possibility that the extent of contraction of collagen lattices by VSMCs is influenced by the collagenolytic activity of these cells and that RPs do not elaborate similar enzymes. However, our observations document that VSMCs are indeed contracting and that the change in the size of the lattice is not due to enzymatic degradation. The contraction of the collagen resulted in a change in the appearance of the lattice from a translucent to an opaque structure, due to an increasingly denser arrangement of the collagen fibrils. When lattices containing no cells were digested with collagenase, they did not shrink in size uniformly nor did they in any way resemble the appearance of lattices contracted by cells. Additionally, conditioned media from cultured cells and media from cells in contracted lattices had no effect on lattices without cells.

The observed differences in VSMC contraction may reflect the state of differentiation of the cells on the different substrates. Chamley-Campbell and Campbell (5) presented morphological evidence that the loss of contractile myofilaments in primary VSMC cultures could be prevented by plating cells at a density that inhibited cell growth. Furthermore, studies by Owens et al. (20) showed that the expression of smooth muscle and nonmuscle isoactins by VSMCs in culture is influenced by their growth state. Synthesis and content of alpha smooth muscle actin was low whereas nonmuscle actins were high in subconfluent cells during exponential growth. Increased synthesis of alpha smooth muscle actin correlated with a decrease in cell proliferation rate, indicating that growth arrest promotes cytodifferentiation. In our studies, VSMCs cultured within collagen matrices showed minimal proliferative activity relative to the growth rates of these cells on plastic or silicone rubber. Thus, in the two assays used, VSMCs could have been in different states of differentiation, which might contribute to variations in their observed contractile ability.

Results obtained from contraction of collagen lattices and silicone rubber clearly show that RPs are significantly more contractile than PMECs. The data that exist concerning contractile proteins in pericytes and ECs support this observation. Joyce et al. (15) have presented evidence that pericytes contain myosin antigenically similar to that of nonmuscle and smooth muscle cells. In contrast, microvessel ECs were shown to stain weakly positive for nonmuscle myosin and appeared to be devoid of smooth muscle myosin. These investigators also reported that pericytes, but not endothelial or other nonmuscle cells, contained smooth muscle tropomyosin (14). Herman and D'Amore (11) have reported that pericytes react with muscle- and nonmuscle-specific antiactins whereas ECs failed to stain with muscle-specific IgGs. Although the specific functions of muscle and nonmuscle contractile proteins within pericytes are unknown, it is clear that the proteins essential for contraction are present in pericytes in higher concentrations than in ECs. These data, and the results reported here, suggest that pericytes are specialized for microvessel contraction, but that endothelial cells may also contribute to microvascular tonus.

Intact actin filaments or stress fibers appear to be part of the mechanism for pericyte contraction. When pericytes were incorporated into collagen lattices, cytochalasin B in-

hibited contraction. When contracted pericytes on silicone rubber were treated with either cytochalasin B or trypsin, the wrinkles in the rubber disappeared indicating a release of tension in both the rubber and the cellular elements causing it to wrinkle. Furthermore, pericytes that wrinkled underlying collagen films always showed microfilament bundles arranged primarily at right angles to the wrinkles, implicating them in the force generated. These results agree with the observations of other investigators supporting a role for stress fibers in force generation (4, 10).

Plasma and serum both stimulate RP contraction suggesting that RPs respond to substances that appear continuously and episodically in blood. For plasma components to influence pericyte contraction, pericytes must have access to these blood-borne mediators. Given the extensive recent documentation that the endothelium plays an active role in the transport of specific metabolites, it is not unreasonable to assume that such a mechanism may exist for the transport of molecules between plasma and pericytes. Whether the plasma factor(s) stimulating RP contraction act directly on pericytes or whether their action is mediated by endothelial cells, however, is not known. Serum contains platelet-release products that are at a higher titer than in circulating blood. However, concentrations similar to those in serum could occur locally at sites of active platelet-vessel interaction. Frequently, a loss of endothelial structural integrity is associated with the platelet release reaction. This permeability dysfunction may provide direct access of platelet-release products to pericytes, which theoretically could alter regional blood flow and permeability.

Pericyte contractility can be effective only if structural mechanisms exist to transmit the force to the underlying vessel wall. Cortoy and Boyles (6) localized fibronectin adhesion plaques in restricted areas between pericytes and ECs, suggesting a mechanical linkage between these two cell types. Our results demonstrate that pericytes contract *in vitro* and that their contraction is greater than that of microvessel ECs. These findings suggest that pericytes may be the principal contributor to the regulation of capillary perfusion *in vivo*.

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