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Sustained orbital shear stress stimulates smooth muscle cell proliferation via the extracellular signal-regulated protein kinase 1/2 pathway

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Objective: **Nonlaminar shear stress stimulates smooth muscle cell (SMC) proliferation and migration in vivo, especially after an endothelial-denuding injury. To determine whether sustained shear stress directly stimulates SMC proliferation in vitro, the effect of orbital shear stress on SMC proliferation, phenotype, and extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation was examined.**

Methods: **Bovine SMCs were exposed to orbital shear stress (210 rpm) for up to 10 days, with and without the ERK1/2 upstream pathway inhibitor PD98059 (10 μM) or the p38 pathway inhibitor SB203580 (10 μM). Proliferation was directly counted and assessed with proliferation cell nuclear antigen. Western blotting was used to assess activation of SMC ERK1/2 and SMC phenotype markers.**

Results: **SMCs exposed to sustained orbital shear stress (10 days) had 75% increased proliferation after 10 days compared with static conditions. Expression of markers of the contractile phenotype (α-actin, calponin) was decreased, and markers of the synthetic phenotype (vimentin, β-actin) were increased. ERK1/2 was phosphorylated in the presence of orbital shear stress, and orbital shear-stress-stimulated SMC proliferation was inhibited in the presence of PD98059 but sustained in the presence of SB203580. Orbital shear-stress-induced changes in SMC phenotype were also inhibited in the presence of PD98059.**

Conclusion: **Orbital shear stress directly stimulates SMC proliferation in long-term culture in vitro and is mediated, at least partially, by the ERK1/2 pathway. The ERK1/2 pathway may also mediate the orbital shear-stress-stimulated switch from SMC contractile to synthetic phenotype. These results suggest that shear-stress-stimulated SMC proliferation after vascular injury is mediated by a pathway amenable to pharmacologic manipulation. (J Vasc Surg 2005;42:772-80.)**

Clinical Relevance: **After an endothelial-denuding injury such as angioplasty, SMCs are directly exposed to hemodynamic forces such as shear stress. Although the exact nature of the shear stress to which the SMCs are exposed in vivo has not yet been defined, the shear-stress forces are likely to be complicated or even turbulent. The orbital shaker produces shear stress that directly stimulates SMC proliferation, suggesting its usefulness as an in vitro model. Because orbital shear-stress-induced SMC proliferation and phenotype modulation is at least partly mediated by the ERK1/2 pathway, shear-stress-stimulated SMC proliferation after vascular injury may be mediated by a pathway amenable to pharmacologic manipulation.**

Although endothelial cells are ordinarily thought to be the only cells exposed to arterial shear stress, smooth muscle cells (SMCs) may also be directly exposed to arterial shear stress after an endothelial-denuding injury such as balloon angioplasty. In addition, interstitial blood flow through the overlying intact endothelial monolayer may expose SMCs to lower magnitudes of shear stress in the absence of a completely denuding injury; in some models, the magnitude of this interstitial blood flow to which SMCs are ordinarily exposed in vivo, in absence of an injury, may approximate arterial magnitudes of shear stress.[1-3]

In vitro laminar flow models have demonstrated that direct exposure of SMCs to laminar shear stress reduces ^{3}[H]thymidine incorporation and proliferation,[4,5] increases basic fibroblast growth factor, angiotensin-converting enzyme, and inducible nitric oxide synthase expression,[6,7] increases cyclic guanosine 3′,5′-monophosphate, tissue plasminogen activator, and nitric oxide production,[8] and increases fibroblast growth factor-2 release.[9] However, the relevance of these in vitro responses of SMCs to laminar shear stress is unclear, as the characteristics of the shear stress to which SMCs are exposed after endothelial cell injury in vivo (ie, laminar versus disturbed or turbulent shear stress) are not clear. Orbital and oscillatory patterns of shear stress in areas of disturbed flow lead to plaque forma-

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tion and vessel remodeling in vivo and activate endothelial cells in vitro.[10,11]

Orbital shear stress activates endothelial cell mitogen-activated protein kinase (MAPK) and pp70^{s6k} pathways and stimulates endothelial cell translation as well as DNA synthesis.[12-14] We have previously demonstrated that short-term exposure to orbital shear stress directly stimulates SMC proliferation and Akt phosphorylation.[15] To determine whether sustained orbital shear stress stimulates SMC proliferation, we examined the activation and functional significance of the MAPK extracellular signal-regulated protein kinase 1/2 (ERK1/2) pathway as a potential mechanism for orbital shear-stress-stimulated SMC proliferation in long term culture in vitro.

MATERIALS AND METHODS

Bovine aortic SMCs were obtained as previously described and maintained in Dulbecco modified Eagle medium F-12 (GIBCO BRL, Gaithersburg, Md) supplemented with 10% fetal bovine serum (FBS), 5 μg/mL deoxycytidine/thymidine (Sigma Chemical, St Louis, Mo), and antibiotics (penicillin, 100 U/L; streptomycin, 100 μ/mL; amphotericin B, 250 ng/mL) at 37°C in a humidified incubator with 5% carbon dioxide.[15]

Cells of passage 4 to 6 were seeded at a subconfluent density of 50,000/cm^2 on six-well plates coated with collagen type I. Cells that were seeded only in the center or only on the periphery of the well were excluded from the unseeded part of the well. To prevent cell attachment, the unseeded area was covered with a silicone gasket that was removed before shear stress exposure. The center area available for seeding was 1.54 cm^2, and 2.92 cm^2 was available in the periphery area.[16]

Cells were exposed to 210 rpm orbital shear stress for up to 10 days using an orbital shaker (DS-500, VWR International, West Chester, Penn), as previously described.[13,15-17] Shear-stress experiments that measured proliferation were performed in the presence of 10% FBS after synchronization by incubation for 24 hours in FBS-free media, and in the additional presence or absence of the MAPK ERK1/2 pathway inhibitor PD98059 (10 μM) or the MAPK p38 pathway inhibitor SB203580 (10 μM). The medium was changed every 2 days. Shear-stress experiments that measured ERK1/2 phosphorylation were performed in serum-free medium to prevent serum activation of the ERK1/2 pathway.

SMC proliferation was assessed by the determination of cell number with a Coulter-Counter (Model ZM, Coulter Electronics, Hialeah, Fla), with some representative samples verified independently with a hemocytometer. Each value was the mean of four counts. SMC morphology was evaluated with hematoxylin and eosin staining. Proliferating cell nuclear antigen (PCNA) staining was evaluated by counting the percentage of positively stained nuclei in five high-power microscopic fields; only definitive nuclear staining was counted.

Western blotting was performed with equal amounts of protein (30 μg) using anti-PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), anti-α-actin antibody, anti-β-actin antibody, antivimentin antibody, anticalponin antibody (Sigma), anti-ERK1/2 antibody, or antiphospho-specific ERK1/2 antibody (Cell Signaling, Beverly, Mass), before detection with enhanced chemiluminescence (Amersham, Piscataway, NJ). All blots were quantified with densitometry (BioImage, Ann Arbor, Mich).

Data were recorded as mean ± SEM and compared with analysis of variance, using the Fisher's post hoc analysis. StatView 5.0.1 software (SAS Institute, Inc, Cary, NC) was used for the calculations. A value of $P < .05$ was considered significant.

RESULTS

Sustained orbital shear stress stimulates SMC proliferation. Bovine aortic SMCs were exposed to sustained orbital shear stress (210 rpm) or static conditions in the presence of 10% FBS and their proliferation was assessed. SMCs exposed to orbital shear stress demonstrated a 35% increase in proliferation by 5 days compared with SMCs cultured under static conditions; by 10 days in culture, SMCs exposed to orbital shear stress had a 75% increase in proliferation compared with those cultured under static conditions (Fig 1, *A*).

Because orbital shear stress generated by the orbital shaker is not uniform across the bottom of the culture well but is reduced in magnitude in the center of the culture well, SMCs were differentially cultured either in the center or in the periphery of the culture well and exposed to orbital shear stress for 10 days (Fig 1, *B*).[16] SMCs cultured exclusively in the periphery of the culture well demonstrated 103% increased proliferation compared with SMCs grown under static conditions ($P = .03$); SMCs cultured exclusively in the center of the culture well demonstrated only 45% increased proliferation compared with static conditions ($P = .28$).

To confirm that the orbital shear-stress-stimulated increase in SMC proliferation in the periphery of the culture well was not an artifact of being differentially cultured exclusively in the periphery, SMCs were seeded throughout the culture well and PCNA expression examined (Fig 1, *C*). SMCs in the periphery of the culture well exposed to orbital shear stress demonstrated a sixfold increase in PCNA expression compared with static conditions ($P = .003$); SMCs in the center of the culture well exposed to orbital shear stress demonstrated only a 2.5-fold increased expression compared with static conditions ($P = .15$). The morphology of SMCs grown under static and orbital shear stress conditions for 10 days is demonstrated in Fig 1, *D*; SMCs in the periphery demonstrate alignment perpendicular to the direction of flow.

Since orbital shear stress stimulates SMC proliferation over 10 days in vitro, we determined whether this increased proliferation was accompanied by a change in SMC phenotype markers. α-Actin, a marker of the SMC contractile phenotype, was decreased in SMCs after 10 days in culture; however, α-actin was significantly decreased (43%) to a greater extent in SMCs exposed to orbital shear stress, with

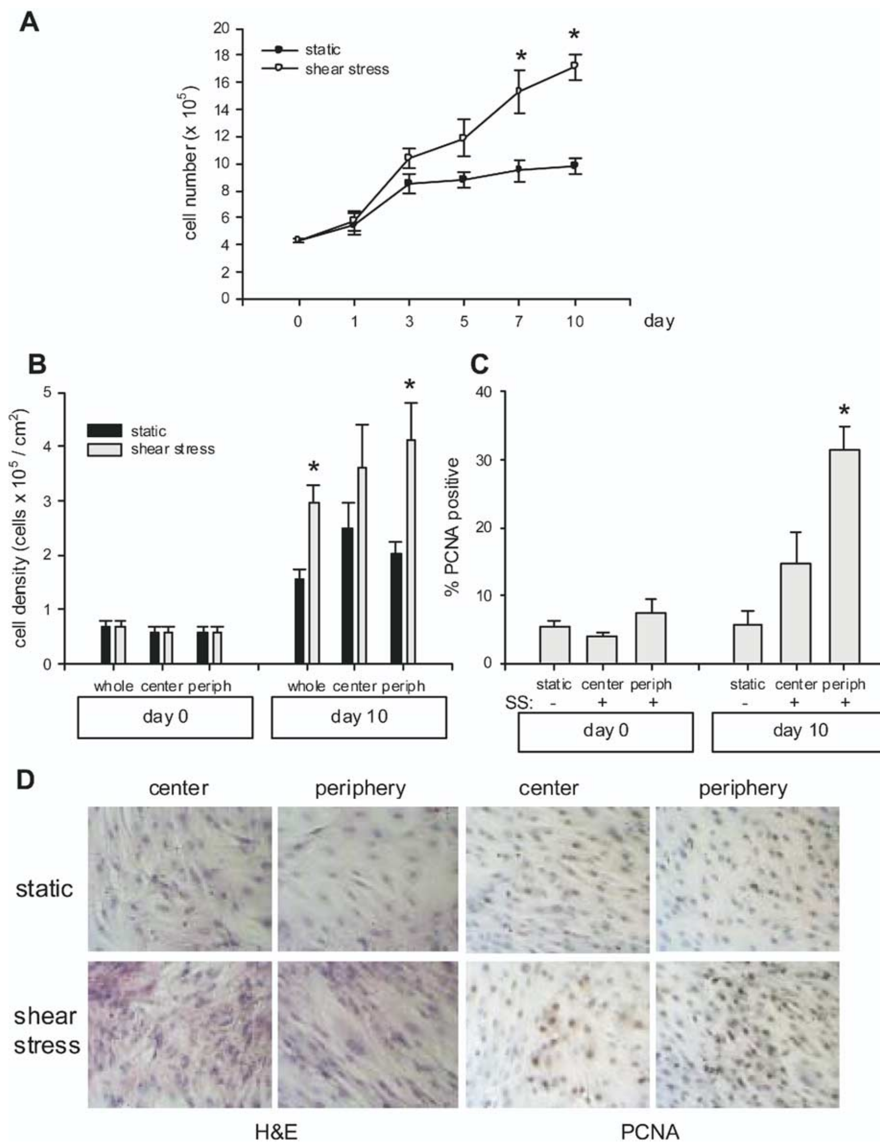

Fig 1. Proliferation and morphology of smooth muscle cells (SMCs) under orbital shear stress. **A,** Line graph shows the time course of SMC numbers for ≤10 days. SMCs exposed to orbital shear stress (○) demonstrated significantly increased cell numbers compared with static (●) conditions (n = 5; $P < .0001$, analysis of variance [ANOVA]; *days 7 and 10, $P = .01$ and $P = .002$, respectively, post hoc). **B,** Bar graph reflects the cell density of cells seeded uniformly in the whole culture well or exclusively in the center or periphery of the culture well. SMCs exposed to orbital shear stress in the whole well or exclusively in the periphery of the well exhibited significantly increased cell density compared with static conditions (n = 4; *$P = .006$ and $P = .03$, respectively, ANOVA). **C,** Bar graph reflects the percentage of cells staining positively for proliferating cell nuclear antigen. SMC exposed to orbital shear stress in the periphery of the well demonstrated significantly increased PCNA expression compared with static conditions (n = 3, $P = .006$, ANOVA; *periphery, $P = .002$, post-hoc). **D,** Morphology of SMCs after 10 days of culture (×300). The edge of the culture well is to the right in all panels. The top panels are under static conditions; the bottom panels are under shear-stress conditions. The left panels are stained with hematoxylin and eosin *(H&E)*; the right panels are stained for proliferating cell nuclear antigen *(PCNA)*. SMCs exposed to orbital shear stress in the periphery of the well were slightly elongated and aligned perpendicularly to the direction of flow.

greater depression in the periphery of the well (64%) (Fig 2, *A*). Similarly, calponin was decreased 18%, with a 22% decrease in the periphery (Fig 2, *B*). Vimentin, a marker of the SMC synthetic phenotype, had 84% greater expression in SMCs exposed to orbital shear stress compared with SMCs cultured under static conditions (Fig 2, *C*). Similarly, β-actin had 53% greater expression in SMCs exposed to orbital shear stress (Fig 2, *D*).

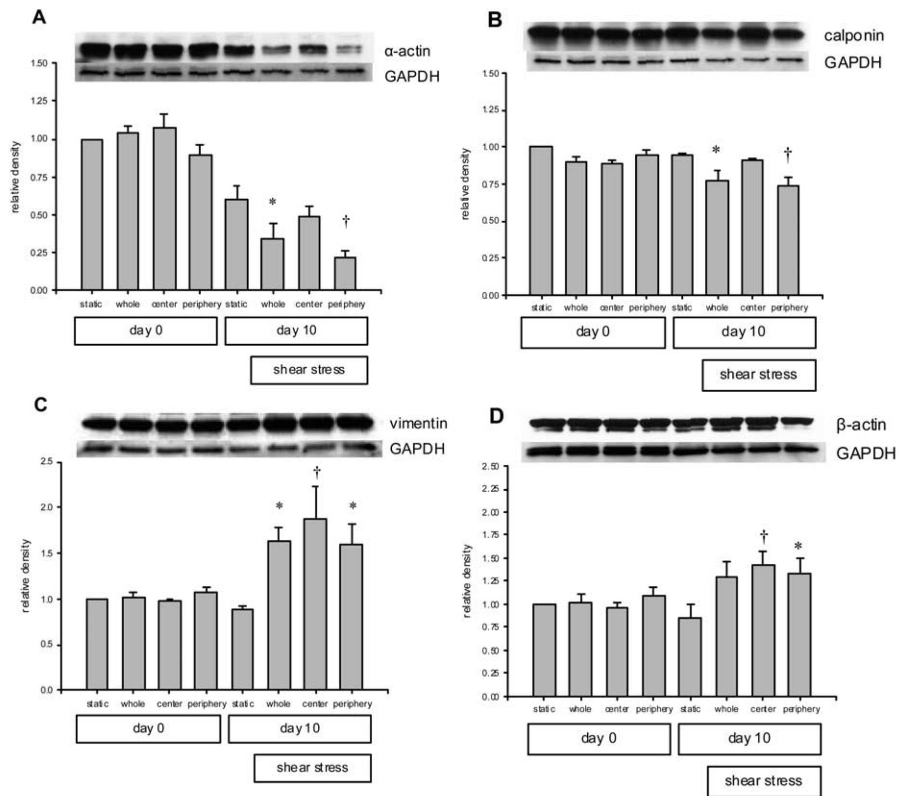

Fig 2. Phenotypic modulation of smooth muscle cells (SMCs) under orbital shear stress. **A,** α-Actin, a marker of the SMC contractile phenotype, had significantly decreased expression in SMCs exposed to orbital shear stress (n = 3; *P* = .04, analysis of variance [ANOVA]; **P* = .04, post hoc), with greater decrease in the periphery of the well (†*P* = .009, post hoc). **B,** Calponin, a marker of the SMC contractile phenotype, had significantly decreased expression in SMCs exposed to orbital shear stress (n = 3; *P* = .03, ANOVA; **P* = .03, post hoc), with a greater decrease in the periphery of the well (†*P* = .01, post hoc). **C,** Vimentin, a marker of the SMC synthetic phenotype, had significantly increased expression in SMCs exposed to orbital shear stress (n = 3; *P* = .05, ANOVA; **P* = .04, post hoc), with increases in both the center and periphery of the well (†*P* = .01 and **P* = .05, post hoc). **D,** β-Actin, a marker of the SMC synthetic phenotype, had increased expression in SMCs exposed to orbital shear stress (n = 4; *P* = .09, ANOVA), with significant increases in both the center and periphery of the well (†*P* = .02 and **P* = .04, post hoc). *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase.

Orbital shear stress stimulates SMC ERK1/2 phosphorylation. To determine a mechanism for orbital shear-stress-stimulated SMC proliferation, the phosphorylation of ERK1/2, a component of the MAPK pathway phosphorylated in response to external proliferative signals, was examined. ERK1/2 was phosphorylated in a time-dependent manner in response to orbital shear stress, with maximum phosphorylation at 15 minutes (Fig 3, *A*). Both basal and orbital shear-stress-stimulated ERK1/2 phosphorylation was inhibited by PD98059, an upstream inhibitor of the MAPK ERK1/2 pathway (Fig 3, *B*). SMCs differentially cultured in the periphery of the well had greater ERK1/2 phosphorylation than those differentially cultured in the center of the well, which was inhibited by PD98059 (Fig 3, *C*). These results suggest that orbital shear stress directly stimulates phosphorylation of the MAPK ERK1/2 pathway in SMCs.

To determine whether SMC ERK1/2 phosphorylation by orbital shear stress plays a role in orbital shear-stress-stimulated SMC proliferation, SMCs were cultured in vitro for 10 days in the presence or absence of PD98059. SMCs cultured in the whole well in the presence of orbital shear stress had increased proliferation compared with SMCs cultured in static conditions, which was inhibited by the presence of PD98059 (Fig 4, *A*); there was no reduction of proliferation in the presence of SB203580, an upstream inhibitor of the MAPK p38 pathway (Fig 4, *B*). Similarly, SMCs differentially cultured in the periphery of the well demonstrated increased proliferation with orbital shear stress, which was inhibited in the presence of PD98059 (Fig 4). These results suggest that ERK1/2 phosphorylation by orbital shear stress is a mechanism for orbital shear-stress-stimulated SMC proliferation.

To determine whether ERK1/2 phosphorylation plays a role in the SMC phenotype change associated with orbital shear-stress-stimulated proliferation, phenotype modulation was examined in the presence of PD98059. Compared with SMCs exposed to static conditions, SMCs exposed to

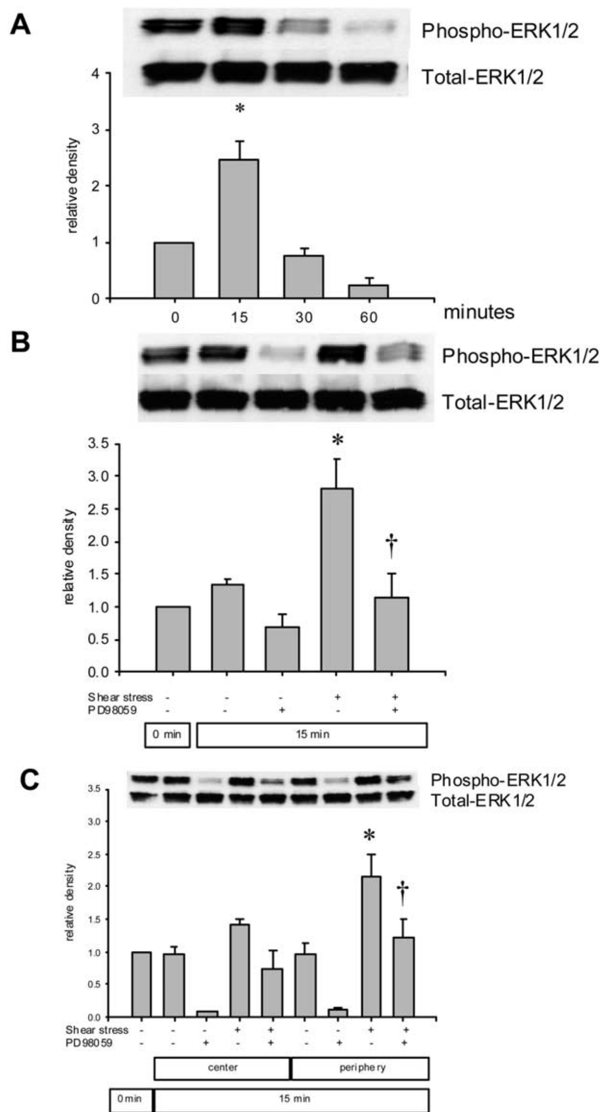

Fig 3. Extracellular signal-regulated protein kinase 1/2 *(ERK1/2)* phosphorylation with orbital shear stress. **A,** Time course of ERK1/2 phosphorylation by orbital shear stress in smooth muscle cells. Bar graph demonstrates maximal ERK1/2 phosphorylation (147%) at 15 minutes (n = 4; $P < .0001$, analysis of variance [ANOVA]; $^*P < .0001$, post hoc). **B,** Inhibition of ERK1/2 phosphorylation with PD98059 (10 μM) (n = 4; $P = .002$, ANOVA). The 110% increase in ERK1/2 phosphorylation is significant ($^*P = .005$, post hoc), as is the inhibition with PD98059 ($^\dagger P = .002$, post hoc). **C,** Inhibition of ERK1/2 phosphorylation with PD98059 in smooth muscle cells differentially cultured in the center or periphery of the culture well (n = 3). The 116% increase in ERK1/2 phosphorylation in the periphery is significant ($^*P = .0003$, post hoc), as is the inhibition with PD98059 ($^\dagger P = .002$, post hoc).

orbital shear stress demonstrated decreased expression of α-actin, which was inhibited by PD98059 (Fig 5, *A*). SMCs cultured differentially in the periphery of the well, but not the center, also demonstrated inhibited α-actin expression with exposure to orbital shear stress, which was inhibited by PD98059 (Fig 5, *B*). Similarly, compared with SMCs exposed to static conditions, SMCs exposed to orbital shear stress demonstrated increased expression of vimentin, which was inhibited by PD98059 (Fig 5, *C*). SMCs cultured differentially in the periphery of the well, but not the center, also demonstrated increased vimentin expression with exposure to orbital shear stress, which was inhibited by PD98059 (Fig 5, *D*).

DISCUSSION

We demonstrate that orbital shear stress directly stimulates SMC proliferation in long-term culture in vitro and that the MAPK ERK1/2 pathway at least partly mediates orbital shear-stress-stimulated SMC proliferation. In addition, the ERK1/2 pathway may also mediate the orbital shear-stress-stimulated change in SMC phenotype markers.

Increased laminar blood flow through vascular grafts is associated with decreased SMC proliferation and neointimal hyperplasia in vivo[18,19]; corresponding in vitro models have demonstrated decreased SMC proliferation with direct exposure to laminar shear stress for 24 hours.[4,5] However, since humans with carotid bifurcation stenosis live for long periods with oscillatory and orbital flow, and since increased SMC proliferation and ERK1/2 phosphorylation have been reported in rat carotid arteries after balloon injury,[20,21] we believe that the orbital shear-stress model is also a physiologically relevant model that more closely approximates the complex, disturbed, and orbital blood flow after an endothelial-denuding injury such as angioplasty than does the laminar flow model. We have previously reported that short-term exposure to orbital shear stress increases SMC proliferation by approximately 20%, and we extend these observations to demonstrate 75% increased SMC proliferation over longer-term culture in vitro (ie, 10 days).[15]

Although the orbital shaker does not produce uniform laminar shear stress on the seeded cells, most of the cells are exposed to near-maximal shear stress (τ_{max}), calculated as

$$\tau_{max} = a\sqrt{\rho\eta[2\pi f]^3}$$

where a is the orbital radius of rotation of the shaker, ρ is the density of the culture medium, η is the viscosity of the medium, and f is the frequency of rotation.[12,13] At 210 rpm, the frequency of rotation used in these experiments, calculated τ_{max} is 9.8 dyne/cm^2 and is similar to the value that we measured (11.1 dyne/cm^2) as well as the values reported by others (11.5 dyne/cm^2) and is similar to arterial magnitudes of shear stress in vivo.[13,16,22]

Because the cells seeded exclusively in the periphery of the culture well have increased proliferation, perpendicular alignment, phenotype change, and ERK1/2 phosphorylation compared with SMCs seeded exclusively in the center of the well, it is possible that the difference between center and periphery effects is due to the reduced magnitude of shear stress in the center of the well (ie, the effects of orbital

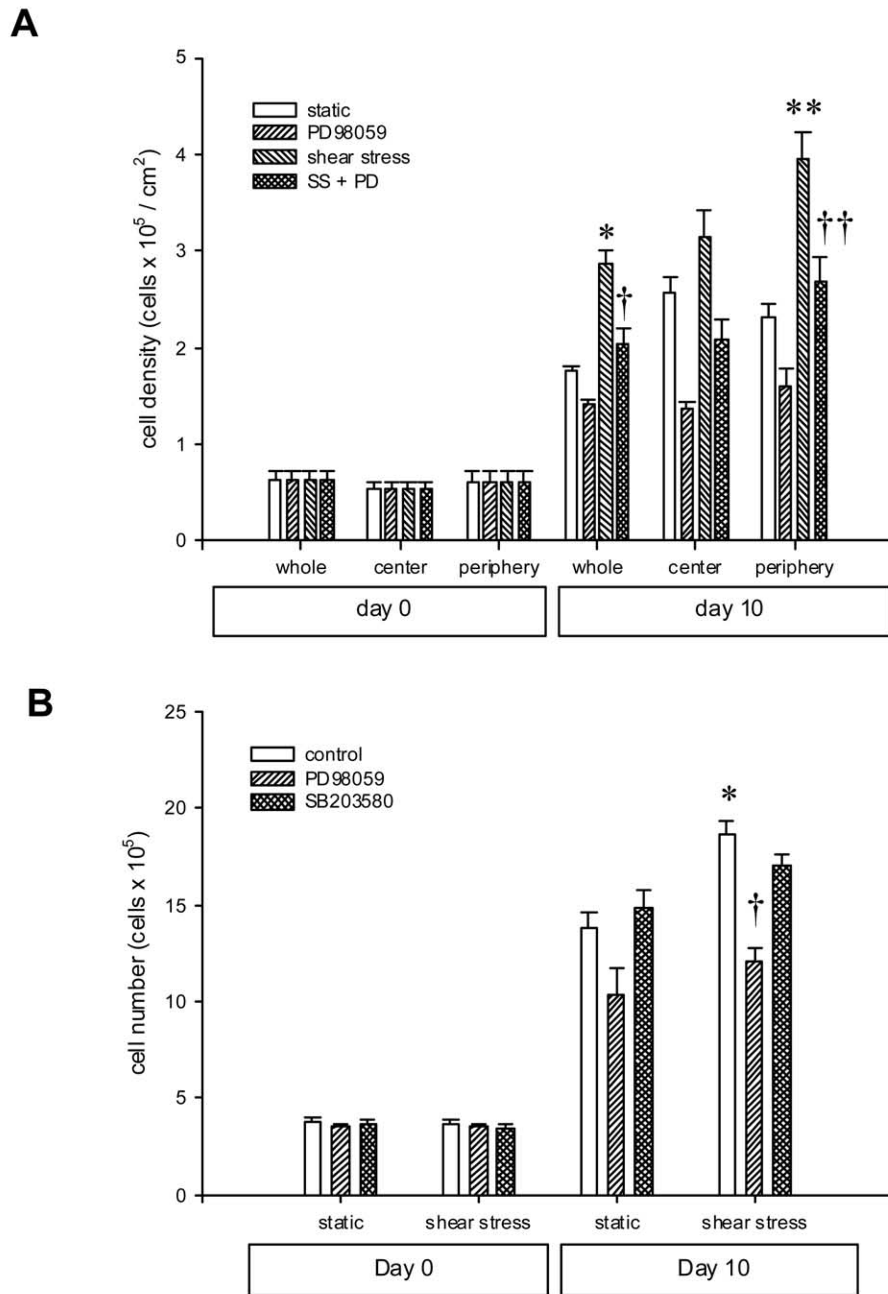

Fig 4. Dependence of smooth muscle cell (SMC) proliferation on extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation. **A,** Bar graph demonstrates SMC density at 0 and 10 days of SMCs cultured in the whole well or differentially cultured in the center or periphery of the well (n = 4). SMCs were cultured in the presence of 10% fetal bovine serum (FBS) and with PD98059 (10 μM) or vehicle alone. For SMCs seeded in the whole culture well, the increase in proliferation due to orbital shear stress is significant ($^*P < .0001$, post hoc), as is the inhibition with PD98059 ($^\dagger P < .0001$, post hoc). For SMCs seeded only in the periphery, the increase in proliferation due to orbital shear stress is significant ($^{**}P = .0002$, post hoc), as is the inhibition with PD98059 ($^{\dagger\dagger}P = .001$, post hoc). **B,** Bar graph demonstrates SMC numbers at 0 and 10 days of SMCs cultured in the whole well, in the presence of 10% FBS and with either PD98059 (10 μM), SB203580 (10 μM), or vehicle alone (n = 4). The increase in SMC proliferation due to orbital shear stress is significant ($^*P = .0008$, post hoc); the reduced proliferation in the presence of the ERK1/2 pathway-inhibitor PD98059 is significant ($^\dagger P < .0001$, post hoc); the effect of the p38 pathway-inhibitor SB203580 was not significant ($P = .20$, post hoc).

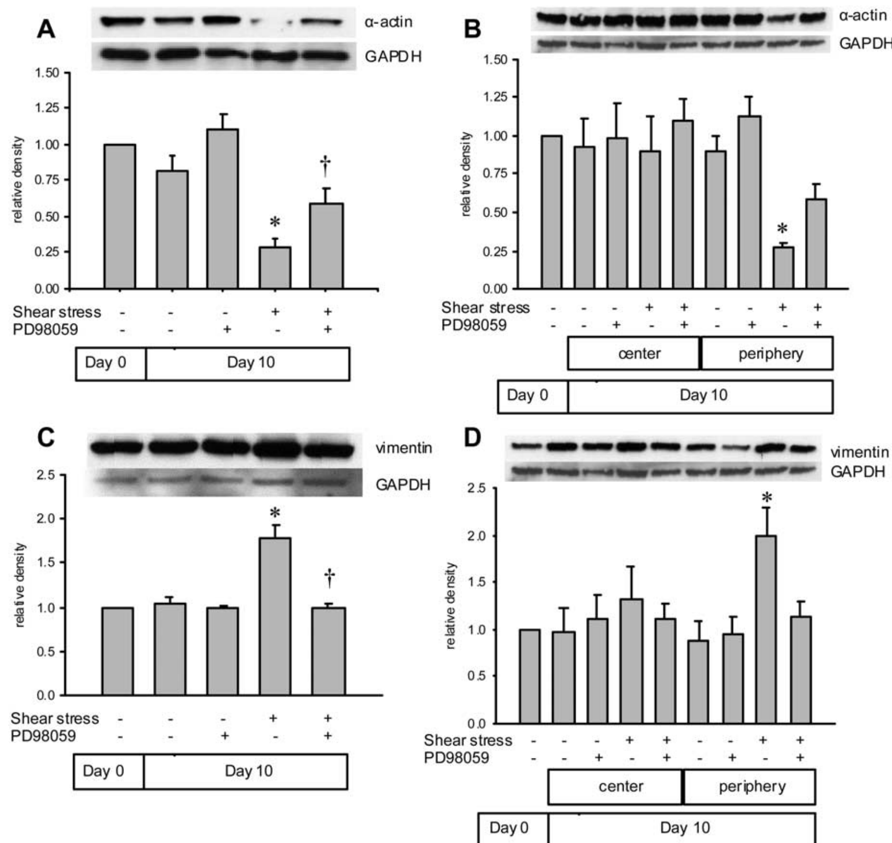

Fig 5. Inhibition of orbital shear-stress-induced smooth muscle cell (SMC) phenotype modulation with PD98059 (10 μM). **A,** α-Actin, a marker of the SMC contractile phenotype, had 71% decreased expression in SMCs exposed to orbital shear stress (n = 4; **P* = .0005, post hoc) but was only decreased 41% in the additional presence of PD98059 (†*P* = .02, difference between orbital shear stress and PD98059, post hoc). **B,** α-Actin expression was reduced 72% in SMCs in the periphery (n = 3; **P* = .009, post hoc), but was only decreased 31% in the additional presence of PD98059 (*P* = .16, difference between control and PD98059, post hoc). **C,** Vimentin, a marker of the SMC synthetic phenotype, had 72% increased expression in SMCs exposed to orbital shear stress (n = 3; **P* < .0001, post hoc) but was decreased 1% in the additional presence of PD98059 (†*P* < .0001, difference between orbital shear stress and PD98059, post hoc). **D,** Vimentin expression was increased 125% in SMCs in the periphery (n = 4; **P* = .002, post hoc) but was only increased 29% in the additional presence of PD98059 (*P* = .44, difference between control and PD98059, post hoc). *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase.

shear stress on SMC are dose-dependent). The exact magnitude of shear stress to which SMCs would be exposed in vivo after an endothelial-denuding injury is not known; thus, the relevance of the exact arterial magnitude of shear stress generated in the periphery of the culture well by this model is not clear. Reduced magnitudes of shear stress, such as present in the center of the culture well, may also be physiologically relevant.

Furthermore, the differences in flow between the center and the periphery of the well in this model may be more relevant when applied on endothelial cells than SMCs because endothelial cells have different types of responses in the center and periphery of the well.[16] However, unlike endothelial cells, SMCs have similar direction but different magnitude responses in the center and periphery of the well (Figs 1 to 5), suggesting that studies of orbital shear stress on SMCs may be relevant even if the entire culture well is totally used, without differential culture.

We demonstrate that orbital shear stress directly stimulates ERK1/2 phosphorylation and that both basal and orbital shear-stress-stimulated SMC proliferation depends, at least in part, on activation of the MAPK ERK1/2 pathway. These findings are consistent with the increased activation of this pathway in response to growth factor stimulation of SMC proliferation.[23-30] ERK1/2 is phosphorylated at very low levels in SMC in vivo, and the overlying endothelial monolayer ordinarily prevents ERK1/2 phosphorylation by physiologic levels of shear stress.[31] ERK1/2 is more closely linked to SMC proliferation than other MAPKs, such as the Jun N-terminal kinase or p38 pathways, consistent with our data demonstrating that orbital shear-stress-induced SMC proliferation specif-

ically depends on the ERK1/2, but not the p38, pathway (Fig 4, *B*).[32,33]

Several strategies, including the use of dominant negative mutants or antisense oligonucleotides, can specifically inhibit ERK1/2 activity and thus inhibit SMC proliferation in vivo.[34,35] We used PD98059, a small, cell-permeable molecule that is a specific inhibitor of MAPK kinase-1 (MEK)[36] because PD98059 does not inhibit p70 S6 kinase, phospholipase C, Raf-kinase, cyclic adenosine monophosphate-dependent kinase, protein kinase C, v-Src, endothelial growth factor receptor kinase, PDGF receptor kinase, or PI3K at the concentrations used in this study.[37-39] Although the use of chemical inhibitors is less specific than some techniques used to inhibit signal transduction, such as the use of dominant negative mutations or the use of cells derived from appropriate knockout mice, PD98059 has similar specificity to antisense oligonucleotides[37]; nevertheless, it is possible that PD98059 inhibits other intracellular signal transduction pathways.

SMCs are heterogeneous; in atherosclerotic-prone arteries or after injury, groups or subgroups of SMCs proliferate and migrate, resulting in pathology such as plaque or restenosis.[40-43] The increase in SMC proliferation and migration is associated with a switch from the contractile to the synthetic phenotype.[44,45] Sustained phosphorylation of ERK1/2 has been demonstrated to play an essential role in the contractile-to-synthetic phenotype switch; culture in vitro was also associated with a decreased number of cellular filaments that was partially reversed by PD98059.[46] Similarly, α-actin expression is decreased in SMCs cultured in vitro.[47] We demonstrate that α-actin expression is decreased in the presence of shear stress beyond that because of static in vitro culture conditions (Fig 2, *A*) and may be partially reversed by PD98059 (Fig 5, *A* and *B*). Similar results were obtained with immunohistochemistry (data not shown).

This suggests that targeting the ERK1/2 pathway to inhibit restenosis not only may inhibit SMC proliferation, but also may preserve the SMC contractile phenotype that is thought to be present in the quiescent arterial wall. However, because the effects of orbital shear stress on SMCs derived from smaller vessels may be different than the effects on SMCs derived from larger vessels, as used in this study, examination of the effects of orbital shear stress on SMCs derived from humans and smaller-diameter vessels may be useful.

We demonstrate decreased α-actin and calponin expression and increased vimentin and β-actin expression with shear stress. Additional markers, such as SM22α, myosin heavy chain, desmin, and smoothelin, may be affected but are not consistently detectable in bovine cells (data not shown).[41] However, taken in toto, our results suggest that shear stress directly affects not only SMC proliferation but also SMC phenotype by the ERK1/2 pathway.

The genes that control the regulation of SMC phenotype are beginning to be understood.[48] If higher level regulatory genes, such as myocardin,[49,50] are responsive to shear stress, then differences in response due to laminar and orbital shear stress may be a potential mechanism for the control of SMC phenotype in vivo. In addition, since SMCs respond directly to shear stress, the shear-stress receptor may be a common feature of both endothelial cells and SMCs.[51,52]

CONCLUSION

Orbital shear stress directly stimulates SMC proliferation and expression of markers of the synthetic phenotype via the ERK1/2 pathway. These results suggest a mechanism for SMC proliferation after an endothelial-denuding injury in vivo and that this response activates a pathway amenable to pharmacologic manipulation.

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