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Increased Synthetic Phenotype Behavior of Smooth Muscle Cells in Response to In Vitro Balloon Angioplasty Injury Model

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Restenosis remains a common problem following balloon angioplasty, and it has been speculated that changes in the mechanical environment due to endovascular interventions are correlated with shifts in smooth muscle cell (SMC) phenotype. In order to study SMC response to forces similar to those exerted during balloon angioplasty, an in vitro concurrent shear and tensile forces simulator has been developed. After 24 hr of exposure to cyclic tension (5%) and shear (0.1-0.5 dynes/cm^2) following simulated angioplasty injury (12% stretch), rat aortic SMCs exhibited significant synthetic behavior. These responses included increased cell proliferation, apoptosis, and cell hypertrophy compared to cells exposed to strain alone. While all SMCs exposed to dynamic stimuli (strain, strain+balloon injury, strain+balloon injury+shear) demonstrated a decrease in contractile protein expression, the injury group also exhibited significantly greater expression of the synthetic marker vimentin. These in vitro findings agree with in vivo events following balloon angioplasty and present a refined dynamic model to be implemented for better understanding of SMC activation and prevention of responses through pharmacological treatment.

INTRODUCTION

Restenosis remains a common problem following balloon angioplasty and stent placement, occurring in 30-60% of cases with balloon angioplasty alone[1] and in 13-36% of cases with stent placement.[2] It is characterized by smooth muscle migration which can begin immediately after balloon angioplasty followed by smooth muscle proliferation in the next 24-72 hr for up to 2 weeks.[3] Increased production of the extracellular matrix and cellular hypertrophy further increases intimal hyperplasia.[4] These initial events of restenosis can be attributed in part to shifts in behavior of medial vascular SMCs from a contractile to a synthetic phenotype.[5] Contractile SMCs are quiescent, morphologically smaller and more elongated than their counterpart; they express relatively higher levels of contractile proteins, which contribute to the cells' ability to contract.[6] In contrast, synthetic SMCs are marked by large morphology, higher cell proliferation and turnover rate, increased extracellular matrix production, and loss of the ability to contract.[6] Thus, blocking of SMC phenotype shift from contractile to synthetic may be a useful way to reduce the incidence of restenosis following balloon angioplasty and has been a focus of research by numerous investigators.[7-11]

While the exact triggering mechanism is yet unknown, it has been speculated that changes in the mechanical environment for SMCs due to angioplasty and placement of stents are correlated with shifts in SMC phenotype. During balloon deployment, both atherosclerotic and healthy SMCs in the medial layer are exposed to higher radial and circumferential forces followed by normally unseen direct shear forces from blood flow

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due to the denudation of the endothelium. In addition, disturbances in blood flow due to atherosclerotic lesions and stent struts can cause further SMC response. Vascular locations with disturbed blood flow are more prone to plaque formation, vessel remodeling, and endothelial cell activation[12-15] and are most frequently associated with intimal thickening leading to restenosis.[3,12,16] Moreover, it has been observed that smooth muscle activation is force- and time-dependent. Studies have shown that intimal thickening results in areas of high shear stress at early time points while low wall shear stress was associated with late intimal thickening, demonstrating negative effects in both cases deviated from an optimum range.[17,18] These results were further substantiated by in vitro studies that demonstrated decreased SMC proliferation when exposed to steady flow[19] and increased SMC proliferation when exposed to oscillatory or orbital shear stress patterns.[7,15,20]

Others have shown that cyclic strain due to the dilation and contraction of blood vessels is just as an important mechanical stimulus as blood flow-induced shear stress to SMC function, such as cell proliferation, migration, apoptosis, morphology, and alignment.[21] While numerous studies have investigated the in vitro SMC response to applied cyclic strain, the results obtained to date vary. Responses can be dependent on parameters such as the SMC vascular location, animal species, and the extracellular matrix[21] as well as on the frequency, amplitude, and time duration exposed to strain. While these studies by other investigators provided many important findings, the models were generally limited to the simulation of either cyclic strain or shear force on SMCs. To simulate the complex arterial mechanical environment that results from endovascular intervention, our lab has designed and fabricated a novel in vitro vascular intervention model which incorporates concurrent shear and tensile forces.[22] Using this setup, we previously demonstrated increased SMC proliferation in response to the concurrent mechanical stimuli after only 8 hours when compared to cyclic strain alone.[22] In addition, our study demonstrated a significant decrease in SM α-actin expression in the concurrent mechanical stimuli group compared to both the static and the cyclic strain only groups.[22] Given these results, we hypothesized that prolonged exposure of SMC to concurrent forces similar to those following balloon angioplasty would result in further synthetic behavior of SMCs. To characterize the SMC phenotype shifts in response to the simulated mechanical environments of endovascular interventions, the present study evaluated cell proliferation, apoptosis, phenotype protein expression, and cell morphology following exposure to the concurrent forces.

MATERIALS AND METHODS

Cell Culture

Rat aortic SMCs (RASMCs) (passages 4-6) harvested from male Sprague-Dawley rats 10-12 weeks old were a generous donation from the laboratory of Dr. Anand Ramamurthi, Department of Bioengineering, Clemson University. RASMCs were cultured in Dulbecco's modified Eagle medium (DMEM, 10-013-CV; Mediatech, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (F-4135; Sigma-Aldrich, St. Louis, MO) and 1% antibiotic-antimycotic (A5955, Sigma-Aldrich) under standard cell culture conditions (37°C, 5% CO_2 with 95% air, humidified environment).

Exposure of RASMCs to Mechanical Stimuli

Our custom-designed, concurrent force, vascular mechanics system was set up and sterilized as previously described.[22] Prior to cell seeding, collagen (50 μg/mL) coated silicone membranes were rinsed with 1.0 mL of sterile Dulbecco's phosphate-buffered saline (DPBS, 21-031 CM; Mediatech). RASMCs were seeded at a density of 2.8×10^4 cells/cm^2 (P4-6) and incubated for 24 hr in DMEM containing 10% FBS and 1% antibiotic-antimycotic. At 24 hr, the medium was changed to DMEM containing 1% FBS, 1% antibiotic-antimycotic, and 1% dextran for an additional 24 hr prior to testing. During mechanical stimulation, RASMCs were maintained in DMEM with 1% FBS and 1% antibiotic-antimycotic; dextran was added to the medium at 1% (w/v) to adjust the media viscosity and resultant wall shear stresses on the cells at the prescribed level.[23]

Three experimental dynamic groups simulating in vivo conditions were investigated in the present study in addition to a static unloaded (U) control group where cells where exposed neither to shear nor tensile forces. Prior to application of mechanical stimuli of interest, the dynamic experimental groups were subject to a preconditioning regimen of 0-4% cyclic strain at gradually increasing frequencies of 0.1 Hz (15 min), 0.5 Hz (15 min), and 1.0 Hz (15 min). The cyclic tensile group (CT) represented a physiological dynamic control and RASMCs were cyclically strained 0-5% at a frequency of 1 Hz for 24 hr. The second group that simulates the clinical balloon angioplasty (BA)