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Unregulated saphenous vein graft distension decreases tissue viscoelasticity

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Abstract

Objectives—Unregulated intraoperative distension of human saphenous vein (SV) graft leads to supraphysiologic luminal pressures and causes acute physiologic and cellular injury to the conduit. The effect of distension on tissue viscoelasticity, a biophysical property critical to a successful graft, is not well described. In this investigation, we quantify the loss of viscoelasticity in SV deformed by distension and compare the results to tissue distended in a pressure-controlled fashion.

Materials and Methods—Unmanipulated porcine SV was used as a control or distended without regulation and distended with an in-line pressure release valve (PRV). Rings were cut from these tissues and suspended on a muscle bath. Force versus time tracings of tissue constricted with KCl (110 mM) and relaxed with sodium nitroprusside (SNP) were fit to the Hill model of viscoelasticity, using mean absolute error (MAE) and r^2 -goodness of fit as measures of conformity.

Results—One-way ANOVA analysis demonstrated that, in tissue distended manually, the MAE was significantly greater and the r^2 -goodness of fit was significantly lower than both undistended

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Author Contribution

Kyle Hocking was involved in the conception and design of the study, data collection, analysis and interpretation of the results, including statistical analysis, as well as writing the article. Eric Wise was involved in the conception and design of the study, analysis and interpretation of the results, including statistical analysis, as well as writing and critically revising the article. Brian Evans was involved in the analysis and interpretation of result and critically revising the article. Craig Duvall was involved in the analysis and interpretation of result and critically revising the article. Joyce Cheung-Flynn was involved in the analysis and interpretation of the results, critically revising the article and obtaining funding. Colleen Brophy was involved in the conception and design of the study, as well as critically revising the article and obtaining funding. All authors gave final approval of the manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Drs. Hocking, Cheung-Flynn and Brophy have a proprietary interest in Vasoprep Inc. Drs. Wise, Evans and Duvall have nothing to disclose.

All research materials can be accessed upon request with the corresponding author.

tissues and tissues distended with a PRV ($p<0.05$) in KCl-induced vasoconstriction and SNP-induced vasodilation.

Conclusions—Unregulated manual distension of SV graft causes loss of viscoelasticity and such loss may be mitigated with the use of an in-line PRV.

Keywords

saphenous vein; bypass grafts; vasoconstriction; viscoelasticity; distension

Introduction

The human saphenous vein (SV) is the most widely used conduit for coronary and peripheral bypass operations in the United States of America. However, patency rates are unsatisfactory, with one-year primary patency reported to be 61% in patients enrolled in the Project of Ex vivo Vein graft Engineering via Transfection (PREVENT) III trial.¹ The primary mechanism of graft failure is neointimal hyperplasia, a complex process that occurs, in part, due to tissue injury. During intraoperative preparation, the graft is first cannulated and then distended with a hand-held syringe to identify unligated branches or missed injuries. Even “gentle” distension with a handheld syringe can lead to intraluminal radial pressures in excess of 600 mmHg.^{2,3} Supraphysiologic distension has been shown to injure the graft. This process severely denudes the endothelial monolayer, which promotes neointimal growth *in vitro*, failure of arteriovenous grafts in a porcine model and increased sensitivity of exposed smooth muscle cells to circulating vasoconstrictors.^{4–6} Deleterious biochemical changes also occur upon distension, including inhibition of prostacyclin generation, increased expression of vascular cell adhesion molecules, induction of the p38 MAPK pathway and release of adenosine triphosphate (ATP).^{3,7–9} Additionally, unregulated manual distension impairs acute physiologic response of the conduit to vasoconstrictive stimuli, including KCl and phenylephrine (PE), and to vasodilatory stimuli, including carbachol and nitroprusside.¹⁰

Pressure distension has been demonstrated to destroy microfibrils and increase the elastic modulus in direct proportion to the applied distension pressure; however, conformity to viscoelastic mechanics has not been well studied.¹¹ In this investigation, a KCl depolarizing stimulus and sodium nitroprusside (SNP) were used to cause vasoconstriction and vasorelaxation, respectively, to determine how porcine SV conforms to a mathematical model of perfect viscoelasticity.¹² We hypothesized that unregulated manual distension would reduce viscoelasticity and deform the SV graft and that this loss of viscoelasticity would yield a sub-optimal conduit that might prove maladaptive to implantation in the arterial tree.

Methods

Procurement of porcine SV

Animal procedures followed protocols approved by the Vanderbilt Institutional Animal Care and Use Committee and adhered to National Institute of Health guidelines for care and use of laboratory animals. The tissue used for this study was obtained after approval by the

Vanderbilt University Institutional Review Board (IRB number M/11/123). Six Yorkshire/Landrace pigs (40 kg) were anesthetized using telazol/ketamine/xylazine and isoflurane maintenance. Subcutaneous fat and fascia were carefully dissected to expose the porcine SV from the ankle, proximal to the sapheno-femoral junction. Branches were ligated using 3-0 silk ties. Once exposed, both SVs were carefully explanted from the body and transferred to the laboratory in heparinized Plasma-Lyte A (HP) (Baxter Healthcare, Mountain Home, AR, USA). Upon harvest, the pigs were euthanized with a sodium pentobarbital overdose.

Distension of porcine SV

Two 1-mm rings of unmanipulated (UM) control tissue were cut from each SV prior to distension. For each pig, one side was randomly designated for unregulated distension and the other for distension with an in-line pressure release valve (PRV) designed to prevent intraluminal radial pressures from exceeding 140 mmHg.¹⁰ The veins were distally cannulated and were fit directly with a 30 mL syringe (unregulated distension) or with an interposed in-line PRV (Figure 1). HP was injected into the segments and the pressure was held for two minutes via clamping of the opposite end.

Physiologic responses to KCl and SNP

Two 1-mm rings from each of the three conditions were physiologically characterized as described previously.¹³ Briefly, the rings were suspended in a muscle bath containing bicarbonate buffer (120 mM NaCl, 4.7 mM KCl, 1.0 mM MgSO₄, 1.0 mM NaH₂PO₄, 10 mM glucose, 1.5 mM CaCl₂ and 25 mM Na₂HCO₃, pH 7.4) equilibrated with 95% O₂ and 5% CO₂ at 37°C for 2 hr. After equilibration, the rings were contracted with 110 mM KCl to determine tissue viability and contractility. The tissues were then re-equilibrated in bicarbonate buffer and precontracted with phenylephrine (PE) (1 μM). PE precontracted tissues were then treated with SNP (0.1 μM) to induce smooth muscle relaxation. Force measurements were obtained at 4 Hz using a Radnoti Glass Technology force transducer (159901A) (Radnoti LLC, Monrovia, CA, USA) interfaced with a Powerlab data acquisition system and Chart software (AD Instruments, Colorado Springs, CO, USA).

Determination of conformity to viscoelastic vasomotion

Raw data of the force generated as a function of time recorded by LabChart was imported into Eureqa Equation Solver (Norton Inc., Somerville, MA, USA) to determine the best fit function. Using symbolic regression, Eureqa is able to find underlying mathematical relationships in datasets. To model the isometric contraction of smooth muscle suspended on the muscle bath apparatus, a Hill elastic model was used. By using force and time as inputs to Eureqa, all necessary variables were available.¹⁴ Viscoelastic conformity was assessed by fitting the tracings of each of the three conditions to the Hill viscoelastic model for isometric contractions of the form $F(t) = (a+rt)/(b+t)$ where the mean absolute error (MAE) and r^2 -goodness of fit were used as primary measures of conformity.¹² In the equation, t is the variable time and a , b and r are constants. A low value for MAE indicates a better fit to a viscoelastic model, as does a greater r^2 -goodness of fit. For each pig, the average MAE and r^2 -goodness of fit between the two rings from each condition were taken for statistical analysis. Representative vasomotor force vs. time tracings with best-fit lines in response to KCl and SNP are illustrated in Figure 2.

Statistical analysis

Data were reported as mean $\pm$ standard error of the mean (SEM). One-way ANOVA among the treatment groups with Tukey's *post-hoc* test with multiple comparisons was conducted in order to determine the level of evidence of differences observed. The criterion for statistical significance was $p < 0.05$. Statistical analysis was performed using GraphPad Prism 5 (La Jolla, CA, USA) and the Eureka Equation Solver.

Results

Contractile response to 110 mM KCl

Six PSVs were contracted with KCl and differences in MAE were found among the treatment groups, as determined using one-way ANOVA ($F_{2,10} = 8.6$, $p = 0.007$). MAE was significantly greater in unregulated distension tissue relative to UM tissue (0.074 ± 0.008 vs. 0.036 ± 0.006 , $n = 6$; Figure 3) and to tissue distended with the PRV (0.074 ± 0.008 vs. 0.045 ± 0.006 , $n = 6$). Differences between UM tissue and tissue distended with PRV were not statistically significant.

Similarly, significant differences in r^2 -goodness of fit were found among the treatment groups ($F_{2,10} = 7.4$, $p = 0.01$). R^2 -goodness of fit was significantly decreased in unregulated distension tissue relative to UM tissue (0.95 ± 0.02 vs. 0.99 ± 0.0008 , $n = 6$, Figure 3) and tissue distended with a PRV (0.95 ± 0.02 vs. 0.99 ± 0.003 , $n = 6$). Differences between UM tissue and tissue distended with the PRV were not statistically significant.

Dilatory response to 0.1 μ M sodium nitroprusside

Three PSVs were dilated with SNP and differences in MAE were found among the treatment groups ($F_{2,4} = 18.0$, $p = 0.01$). MAE was significantly greater in unregulated distension tissue relative to UM tissue (0.087 ± 0.02 vs. 0.025 ± 0.01 , $n = 3$; Figure 4) and to tissue distended with the PRV (0.087 ± 0.02 vs. 0.017 ± 0.009 , $n = 3$). Differences between UM tissue and tissue distended with the PRV were not statistically significant.

Similarly, significant differences in r^2 -goodness of fit were found among the treatment groups ($F_{2,4} = 11.3$, $p = 0.02$). R^2 -goodness of fit was significantly decreased in unregulated distension tissue relative to UM tissue (0.94 ± 0.02 vs. 0.99 ± 0.005 , $n = 3$, Figure 4) and tissue distended with a PRV (0.94 ± 0.02 vs. 0.995 ± 0.001 , $n = 3$). Differences between UM tissue and tissue distended with the PRV were not statistically significant.

Discussion

Viscoelastic tissue, upon contraction or relaxation, has an elastic, recoverable component and a viscous, non-recoverable component. Viscoelasticity is a property of vascular tissue that is essential to prevent continuous deformation upon the application of a constricting or relaxing external force. The viscous component allows for the applied force to level off and plateau, reflecting a dissipation of energy over the course of the application of the load. In effect, the stiffness of a viscoelastic material depends on the rate at which it is being deformed.^{15,16} The importance of robust viscoelastic conformity in a conduit is recognized

in vascular surgery, evident by the insistence of viscoelasticity as an essential biomechanical property inherent in synthetic conduit design.^{17,18}

Viscoelasticity of veins is a vastly understudied area necessitating future research, particularly in contrast to arteries. However, as SVs are frequently used as conduits in both cardiac and peripheral arterial systems, an improved understanding of venous viscoelasticity and strategies to its preservation is necessary. Veins are thinner walled than their arterial counterparts, but contractile function and response to stressors are still primarily governed by tone and quantity of smooth muscle cells, critical proteins of the extracellular matrix and cell-extracellular matrix interactions.¹⁹

Very recent studies correlating the degree of viscoelastic health to clinical outcomes have been explored using the human carotid artery. Specifically, a ratio of gradients of deformation of the vascular wall over a cardiac cycle allowed for measurement of carotid artery viscoelasticity in almost 400 patients, generating an index corresponding to viscoelastic conformity.²⁰ Remarkably, this index was found to be an independent predictor of coronary artery disease.²⁰ This promising clinical data, along with the well-characterized loss of viscoelasticity in arteries associated with hypertension, aging and other pathologic states, suggests that impairment of viscoelasticity may have translatable clinical consequences.¹⁹ These consequences are ostensibly more pronounced in the case of viscoelastic disruption of a venous graft with a more fragile microstructure and smooth muscle cell layer, further exacerbated by its autotransplantation into an arterial system with a likely high burden of baseline vascular disease.¹⁹

Recently, our laboratory showed that standard intraoperative manipulations of human SV led to smooth muscle and cellular dysfunction, increased generation of reactive oxygen species and accelerated intimal growth in an organ culture model, relative to unmanipulated tissue. The manipulated tissue underwent other forms of intraoperative injury besides distension, including preservation in acidic normal saline solution and “off-label” marking with a surgical skin marker.²¹ To determine the contribution of manual distension to these injuries, our laboratory demonstrated that distended SV were functionally impaired and that this impairment was not observed in tissue distended with the PRV in place. Additionally, endothelial-dependent relaxation was reduced in distended SV, but not in SV distended with the PRV, indicating that distension using the PRV preserved the critical endothelial monolayer.¹⁰

Our laboratory further reported the accelerated intimal growth in organ culture observed in acute distension injury in porcine SV. Both control tissue and PRV-distended tissue exhibited minimal increase in intimal thickness; however, fully distended tissue demonstrated comparatively greater neointimal formation.¹⁰ Loss of viscoelasticity (Figures 3 and 4) due to unregulated distension is an additional manifestation of this injury. This loss of viscoelasticity may be due to disruption of the vessel microarchitecture and may render the conduit maladaptive toward interposition into the arterial system and potentially contribute to poor vein graft performance.

In the current investigation, a PRV was used to limit intraluminal pressures to 140 mmHg. Other methods of intraoperative distension have been described. Notably, a cannulated SV can be attached to a side-arm of a standard cardioplegia/vent catheter secured in the aorta during cardiac bypass. This allows for the patient's own arterial pulsation of heparinized blood to distend the vein and has been reported to provide effective visualization of leaks and unligated side branches.²² Despite recognition that pressure-limited distension mitigates conduit injury, manual distension with unregulated pressure remains a prevalent technique, probably due to the lack of simple, readily incorporated technologies available to limit intraluminal pressure.

This study had several limitations. First, due to the need for conduit length, porcine SV was used instead of human tissue. This substitution necessitated a more restricted sample size in accordance with practice guidelines for live animal models. However, porcine SV represents homogeneous, young and healthy tissue and probably has higher conformity to viscoelasticity at baseline, thus, allowing clearer observation and delineation of the effects of distension and the use of the PRV. Next, while care was taken to handle the porcine SV carefully, minimal injury during the explantation procedure and brief period of preservation in HP was inevitable.²¹ The contribution of these factors toward loss of viscoelasticity is currently unknown and will be examined in future studies. Finally, this study measured acute changes in viscoelasticity. Upon implantation, the SV graft will remodel due to the higher pressures seen in the arterial system.

Conclusions

Unregulated manual pressure distension of a saphenous vein conduit diminished its viscoelasticity to KCl and SNP according to the Hill model. Viscoelasticity is a highly desirable biophysical property of conduits because of the ability to properly dilate with arterial flow as laminar flow is beneficial in the prevention of intimal hyperplasia.²³ The loss of viscoelasticity was mitigated by using a PRV during intraoperative preparation, a simple intervention that may, plausibly, improve long-term graft performance, though further studies involving animal survival models are necessitated to definitively demonstrate this.

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Figure 1. Pressure release valve (PRV) with adaptor tubing. Manual distension through an in-line PRV limits radial pressures in the lumen to 140 mmHg.

Figure 2.

Representative force vs. time tracings. **A-** Upon contraction with 110 mM KCl, the force vs. time tracing (blue line) demonstrated an excellent fit to the Hill model of the form $F(t) = (a + rt)/(b + t)$ in tissue distended with a PRV (red line). **B-** Upon challenge with 110 mM KCl, tissue distended without pressure control (blue line) demonstrated a poorer fit to the Hill model (red line). **C-** Upon pre-contraction with phenylephrine (PE) and relaxation with 0.1 μ M SNP, the force vs. time tracing demonstrated an excellent fit to the Hill model in tissue distended with a PRV. **D-** Upon pre-contraction with PE and relaxation with 0.1 μ M SNP, the force vs. time tracing demonstrated a poorer fit to the Hill model.

Figure 3.

Viscoelasticity of porcine SV during vasoconstriction in response to 110 mM KCl. Mean absolute error (MAE; black bars) and r^2 -goodness of fit statistics (gray bars) were calculated to assess viscoelastic conformity to the Hill model. Unregulated distension tissues demonstrated increased MAE and lower r^2 -goodness of fit statistics relative to UM tissue and tissues distended with a PRV. * $p < 0.05$ relative to MAE of unregulated distension tissues, # $p < 0.05$ relative to r^2 -goodness of fit of unregulated distension tissues.

Figure 4.

Viscoelasticity of phenylephrine pre-contracted porcine SV during vasorelaxation in response to SNP. Mean absolute error (MAE; black bars) and r^2 -goodness of fit statistics (gray bars) were calculated to assess viscoelastic conformity to the Hill model. Unregulated distension tissues demonstrated increased MAE and lower r^2 -goodness of fit statistics relative to UM tissue and tissues distended with a PRV. * $p < 0.05$ relative to MAE of unregulated distension tissues, # $p < 0.05$ relative to r^2 -goodness of fit of unregulated distension tissues.