THE EFFECTS OF SHEAR STRESS AND PRESSURE ON SAPHENOUS VEIN REMODELING EX VIVO

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ABSTRACT

We have subjected pig saphenous veins to several ex vivo hemodynamic environments for a period of one week in an effort to investigate the effects of shear stress and pressure on their remodeling, specifically changes in vessel size and biomechanical properties. In general, we observe that veins exposed to the lowest levels of shear stress exhibit the greatest intimal area, 0.23+/-0.07 mm^2, which is significantly different from the intimal areas of freshly harvested veins and veins exposed to elevated flow rates and static conditions, 0.05+/-0.01 mm^2, 0.12+/-0.02 mm^2 and 0.08+/-0.01 mm^2 ($p<0.05$), respectively. Furthermore, veins exposed to higher pressures have greater medial areas (1.46+/-0.09 mm^2) than freshly harvested veins and those exposed to lower pressures, 1.05+/-0.07 mm^2 and 1.2+/-0.06 mm^2 ($p<0.05$), respectively. Lastly, cultured veins are less compliant than fresh saphenous veins; however, veins subjected to elevated flow rates and either venous or elevated pressures have smaller elastic moduli (1.4+/-0.3E6 dyne/cm^2 and 2.6+/-0.3E6 dyne/cm^2, respectively) than those cultured under venous levels of flow and pressure, 17.0+/-11.7E6 dyne/cm^2 ($p<0.05$).

INTRODUCTION

While numerous researchers have developed ex vivo perfusion systems to study the effects of various mechanical environments on saphenous vein grafts, many of these studies have been short-term (hours to days) and have focused on acute changes that occur in the vein [1]. Fewer studies have looked at chronic changes, namely changes in the size or structure of the vein, or vein remodeling, in response to different mechanical environments. Our laboratory has developed an ex vivo perfusion system to subject pig saphenous veins to various physiological, hemodynamic environments in order to study the effects of shear stress and pressure on the chronic remodeling of veins. In addition to analyzing changes in vein size and structure, we have evaluated changes that occur in the biomechanical properties of the veins.

METHODS

Perfusion system

The ex vivo perfusion system has been described in detail previously [2]. A peristaltic pump drives oxygenated medium through the vessel, and pressure in the system is controlled by adjusting needle valves upstream and downstream of the vessel. The entire system, except for the pump, is maintained at 37°C. Pressure transducers are positioned immediately before and after the vessel, and a Laser system is used to dynamically measure the outer diameter of the perfused vessel. Data is digitized and recorded on a personal computer.

Vessel preparation and perfusion conditions

Porcine saphenous veins are harvested from anesthetized pigs (25-35 kg) and transported in sterile medium to the laboratory. Under a laminar flow hood, veins are cannulated on stainless steel tubing and mounted in a Plexiglas vessel chamber at their in vivo length and then installed into the perfusion system.

Saphenous veins are exposed to the following mechanical environments: 1) "Venous" conditions (n=3) – 10 mL/min, 25 mm Hg, 2) "Elevated Flow" conditions (n=3) – 40 mL/min, 25 mm Hg, 3) "Ramped Pressure" conditions (n=4) – 10 mL/min, pressures ramped linearly from 10 to 75 mm Hg over the week of the experiment, 4) "Constant Shear Stress" conditions (n=4) – 10 dyne/cm^2, pressures ramped linearly from 10 to 75 mm Hg over the week of the experiment. Rings of veins are also cultured for one week in a static environment ("Static"). Freshly harvested saphenous veins ("Fresh") serve as controls.

Upon completion of the experiment, samples of veins are fixed and processed for morphologic staining. Area measurements are made from sections of veins stained for elastin.

Biomechanical testing protocol

Following the work of Cox [3], a pressure head of approximately 80 mm Hg is generated and the vein is allowed to incubate for 20 minutes at this transmural pressure. At the end of this period, the vein is subjected to 10 continuous cycles of inflation and deflation at a rate

of 2 mm Hg/sec between 0 and 100 mm Hg, and then three inflation responses are recorded at 1 mm Hg/sec between 0 and 100 mm Hg. At the end of the experiment, veins are removed from the vessel chamber, blotted lightly with gauze, and weighed.

We assume the vein wall is an incompressible cylinder and calculate the inner radius, R_i, of the vein at each measured outer radius. Internal radii are calculated at each 10 mm Hg step increase in the 10 to 100 mm Hg transmural pressure range. The compliance is calculated as the change in lumenal area induced by a transmural pressure change of 20 mm Hg. The elastic moduli of the veins are also estimated as the slope of the stress-strain curves in the pressure range of 10 to 50 mm Hg. Stress is defined as the transmural pressure multiplied by the ratio of the internal radius to wall thickness and strain as $(R_i-R_{10})/R_{10}$, where R_{10} is the internal radius at 10 mm Hg.

RESULTS

Fig. 1 shows the trend of decreasing intimal area with exposure to greater levels of shear stress during the week of culture. An asterisk represents a statistically significant difference from fresh saphenous veins based on a student t-test with $\alpha<0.05$.

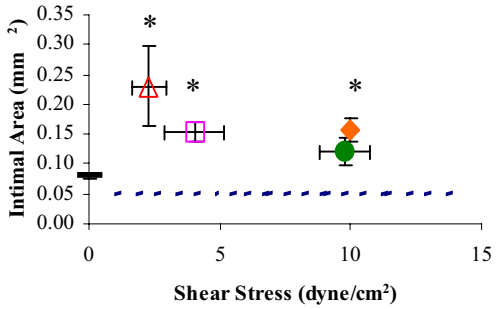

Figure 1. Intimal area vs. shear stress for venous (open pink square), ramped pressure (open red triangle), elevated flow (closed green circle), constant shear stress (closed orange diamond), and static (black dash) conditions. The blue dashed line represents the average intimal area for fresh veins.

In addition, veins exposed to ramped pressure conditions have greater medial areas (1.46+/-0.09 mm^2) than freshly harvested veins and those exposed to lower pressures, 1.05+/-0.07 mm^2 and 1.20+/-0.06 mm^2 ($p<0.05$), respectively.

Figure 2 shows the reduced compliance of cultured veins relative to fresh veins.

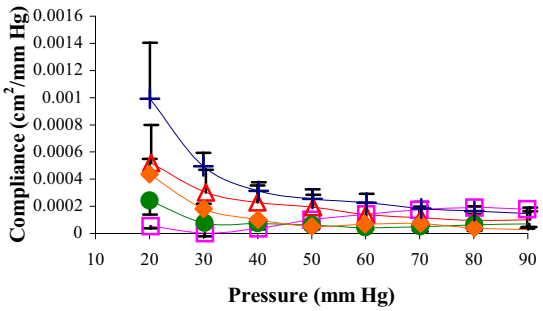

Figure 2. Compliance vs. pressure for fresh saphenous veins (blue cross), venous (open pink square), ramped pressure (open red triangle), elevated flow (closed green circle), and constant shear stress (closed orange diamond) conditions.

Figure 3 shows the increased stress developed in veins perfused under venous conditions relative to other culture conditions.

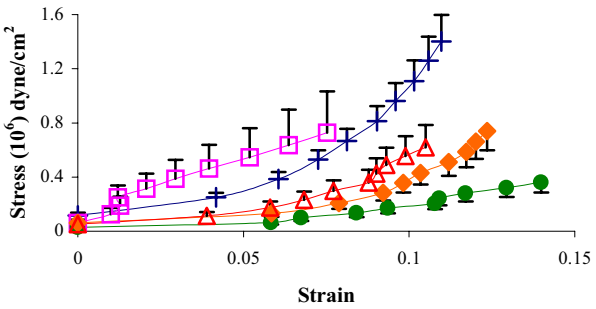

Figure 3. Stress vs. strain (symbols as defined for Figure 2).

The elastic modulus is greatest for those veins cultured under venous conditions (17.0+/-11.7E6 dyne/cm^2), which is significantly greater than the moduli of veins subjected to elevated flow rates and either venous or ramped pressures, 1.4+/-0.3E6 dyne/cm^2 and 2.6+/-0.3E6 dyne/cm^2 ($p<0.05$), respectively.

DISCUSSION

Trends in our data suggest that after one week of ex vivo culture, pig saphenous veins remodel in response to their mechanical environment. Intimal areas were greatest for those veins exposed to the lowest levels of shear stress, while medial areas were greatest for those veins exposed to higher pressures. These results are consistent with observations of saphenous vein grafts made in vivo, in which the development of intimal hyperplasia has been related to low shear stress and medial thickening has been related to elevated pressure. Furthermore, it appears that in addition to changes in vessel size, changes in vessel biomechanics can also be induced by chronic changes in mechanical environment. Such a system may be useful for both studying the effects of mechanical environment on vein graft failure, as well as investigating the use of the mechanical environment to tissue engineer grafts for bypass surgery.

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