Particle Deposition in Arteries *Ex Vivo*: Effects of Pressure, Flow, and Waveform

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The goal of this study was to quantify the effect of hemodynamic pressure, flow and waveform perturbations on the deposition of protein-sized particles in porcine carotid arteries ex vivo. An ex vivo perfusion system was used to control the pressure and flow environment for excised arterial tissue. Confocal laser microscopy images revealed that 200 nm particles were deposited intimally and that more spheres were evident along vessels perfused under oscillatory waveform conditions than all others. Under all pressure, flow and waveform conditions, particles were excluded from the media and adventitia of the vessel wall. The steady flow data support the use of Darcy's Law with pressure-dependent hydraulic permeability to model arterial tissue. [DOI: 10.1115/1.1572905]

Introduction

Throughout the cardiovascular system, blood flow and pressure are unsteady and pulsatile, with oscillatory (zero-mean) flow in areas of bends and bifurcations [1]. The mechanical forces generated by physiological blood flow in compliant vessels include cyclic stress and cyclic strain throughout the wall and time-varying fluid shear stresses along the intima. These solid wall stresses and strains, and fluid shear stresses are both temporally and spatially heterogeneous [2,3]. Characteristics of these mechanical forces and their combination have been postulated as initiating factors in atherogenesis. Since the accumulation of plasma proteins such as low-density lipoprotein (LDL) in the artery wall is an important component of atherogenesis [4], the effects of biomechanical forces on arterial wall permeability to these proteins is important. Specifically, the ratio of inner wall stress to outer wall stress [2], cyclic strain [5], low and oscillatory fluid shear stress [3], permeability changes due to shear stress [6,7], LDL incorporation and metabolism changes due to shear stress [8], and the combination of solid wall strain and low shear stress [9] have been implicated in disease initiation. Biochemical stimuli have also been explored as primary events in the development of atherosclerosis; for example, elevated LDL levels have been shown to increase endothelial cell (EC) permeability to LDL, which may begin a cascade of pathological events [10].

In order to distinguish between these various theories for disease development, it is necessary to separate the heterogeneous effects of fluid shear stress from those of solid wall stress and strain. It is also useful to separate these mechanical stimuli from biochemical and immunological factors. In this way, the contributions of each category of stimulus to vascular cell structure and function change and resulting atherogenic increases in vascular permeability can be independently determined. Thus, in the absence of spatially-varying solid wall stresses and biochemical and immunological factors, the effects of flow, waveform and mean pressure on the intimal deposition of large, inert particles in straight sections of porcine carotid arteries were investigated.

Straight vessels with only very slight taper were used to ensure a homogeneous solid wall strain distribution in the tissue. Carboxylate-modified polysterene beads were used to determine susceptibility to large protein accumulation in the absence of complicating receptor-mediated or charge polarization effects. In addition, fluorescent dye within the beads facilitated easy visualization by fluorescent or confocal laser microscopy. Large particles, the size of plasma proteins such as chylomicrons or triglycerides (approx. 200 nm diameter) [11], were chosen to investigate intimal permeability changes to large particles and estimate vessel permeability to fluid flux. An *ex vivo* perfusion system was used to control the waveform, pressure and flow environment for live arterial tissue. The *ex vivo* system also has the advantage of controlling the biochemical environment so that it is consistent across all experimental conditions, and precludes circulating immune and coagulation factors from affecting the results.

Three waveforms (steady, pulsatile and oscillatory) and two mean pressure levels (100 and 200 mm Hg) were tested for their effects on particle deposition in live porcine carotid arteries. Control vessels were perfused under identical pressure but zero mean, very low amplitude flow conditions to test for the effect of flow (presence or absence). Our hypotheses were (1) that increasing pressure would increase particle deposition by Darcy's Law and since hypertension is associated with atherogenesis and greater intimal lipid accumulation *in vivo*, and (2) that flow (presence versus absence) and waveform (steady versus pulsatile versus oscillatory) would affect particle deposition. In particular, we hypothesized that the oscillatory waveform would lead to the most uptake of particles since atherogenic protein accumulation occurs most frequently at sites of oscillatory flow *in vivo*.

Methods

Common carotid arteries were harvested from Landrace-Yorkshire cross pigs obtained from a local farm (Cambridge VT, average weight 105 ± 30 lbs). Sterile techniques were used to harvest and store arteries until use (always less than 16 hours). Previous studies have demonstrated good viability of endothelial and smooth muscle cells in vessels harvested in this way [12]. However, a comprehensive assessment of cellular viability was not performed in this study. Straight, non-branching sections of artery 6 ± 2 cm in length were dissected from the carotid sheath. From each isolated vessel, two equal halves were prepared for *ex vivo* perfusion as experiment and control. The experimental artery section was sutured into a vessel chamber filled with Dulbecco's Modified Eagle's medium (DMEM, Sigma Co., St. Louis, MO). The control vessel was sutured into a separate chamber connected in a T-configuration to the main flow loop (See Fig. 1). During all experiments, perfusate (DMEM with 5% dextran added to match whole blood viscosity, $\mu = 4.0$ cP) and chamber bathing media were supplied with 5% CO_2 and 95% air for proper pH balance and maintained at 37°C. Steady flow was generated with computer control (LabView, National Instruments, Austin TX) of a roller-pump (MasterFlex, Cole Parmer, Vernon Hills, NJ) with two in-

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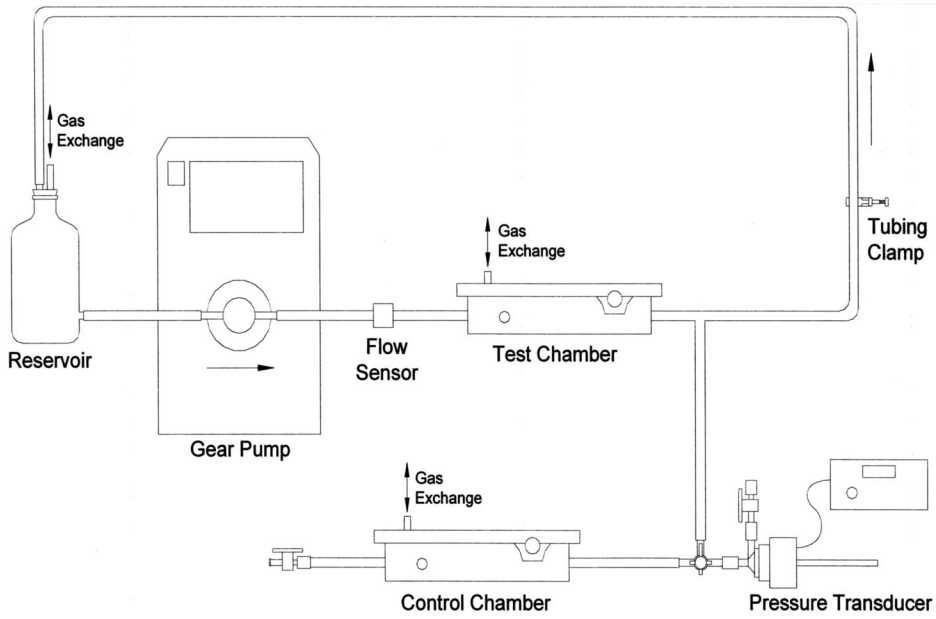

Fig. 1 *Ex vivo* perfusion system for pulsatile waveform, variable pressure.

line pulse dampeners consistent with previous studies [13]. Pulsatile and oscillatory waveforms were generated with computer control of a gear pump (Ismatec, Cole Parmer, Vernon Hills, NJ) that had a faster response time and did not generate roller-induced flowrate fluctuations (Fig. 1). The perfusion system for oscillatory flow was identical to the pulsatile one except that independent, pressurized reservoirs stopped flow upstream of the pump and downstream of the vessel. To obtain a physiological mean shear stress of 15 dynes/cm^2 for vessels of average inner diameter 4.5 mm under steady and pulsatile conditions, the mean flow rate was set to 210 ml/min. The mean flow rate for the oscillatory waveform was approximately zero. The amplitude of shear stress oscillation for the pulsatile and oscillatory waveforms was set to 4 dynes/cm^2 by controlling the flow waveform at the gear pump. This value was chosen to approximate the amplitude of shear oscillation found at the outer wall of the carotid sinus in humans (-1.5 ± 5.5 dynes/cm^2) [3]. The mean transmural pressure for all experiments was either 100 or 200 mm Hg, adjusted with a tubing clamp on the downstream side of the vessel. For these conditions, the longitudinal gradient in transmural pressure within a smooth-walled vessel should be less than 1 mm Hg, assuming Poiseuille-type viscous losses. Time-varying pressures and flow rates were recorded at several time points during each experiment. Representative time-varying flowrate and pressure for the steady and pulsatile waveforms at mean pressure of 100 mm Hg are shown in Fig. 2.

In their respective sealed chambers, experimental and control vessels equilibrated to pressure, flow and *ex vivo* environmental conditions (temperature, pH, etc.) for two hours. Then, a dilute solution of 200 nm Texas red-labeled microspheres (0.1 percent solid fraction in 10 ml, Molecular Probes, Eugene, OR) was added to 400 ml of pefusing media. The experimental vessel was then perfused for six hours with steady, pulsatile or oscillatory flow; the no-flow control vessel experienced pressure fluctuations but no mean flow for the same amount of time, with the same perfusate. The control vessel under oscillatory, no-flow conditions experienced very small fluctuations in shear stress (approx. $<\pm1$). The outlet of the control vessel was bled once each hour to maintain pH and particle concentrations in the vessel lumen. After eight hours total of perfusion, vessels were fixed overnight at pressure in 3 percent paraformaldehyde in phosphate buffered saline (Sigma Co.), flash frozen in tissue freezing medium and stored at -70°C.

Thin (10 μm) were cut and stained with SYTOX green nucleic acid stain (Molecular Probes) for visualization with a Bio-Rad MRC 1024ES confocal laser scanning microscopy system (Bio-Rad Laboratories, Hercules, CA). All images were collected at 10× magnification. Sequential images were captured under 488 nm and 568 nm wavelength excitation to visualize SYTOX green and Texas red fluorescence, respectively. These gray scale images were then imported into Adobe Photoshop (Version 4.5; Adobe Systems, San Jose, CA), colorized and merged as 24-bit RGB. In

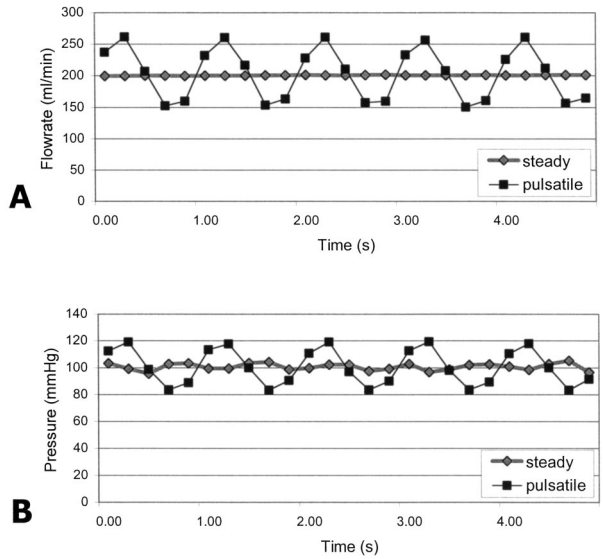

Fig. 2 Representative flowrate (A) and pressure (B) generated during steady and pulsatile perfusion of a porcine carotid artery *ex vivo* at a mean pressure of 100 mm Hg.

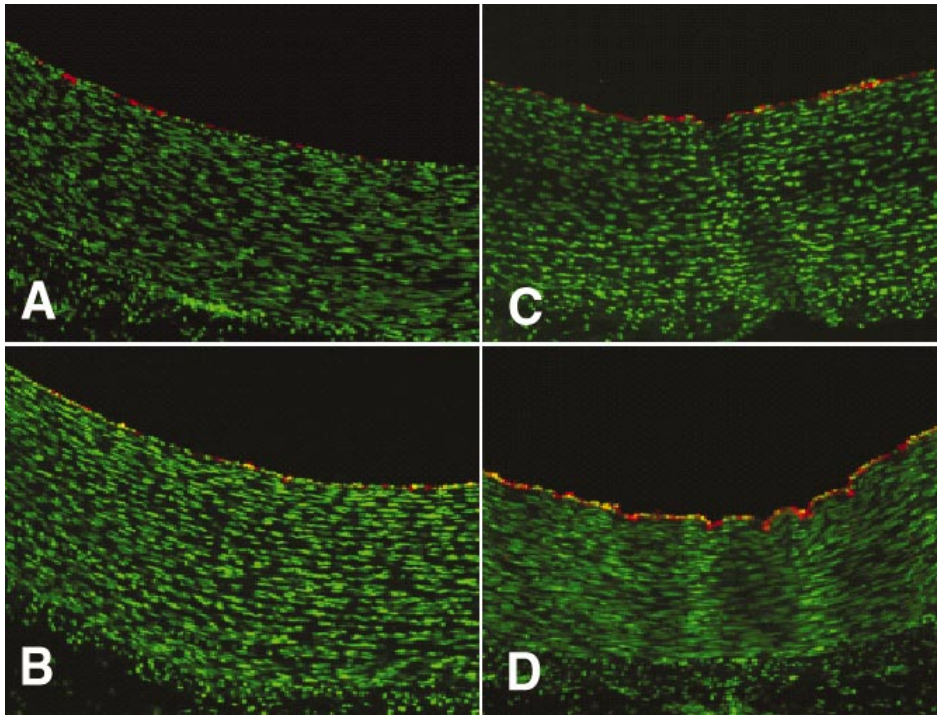

Fig. 3 Confocal laser microscopy images of vessels perfused with microspheres under steady (A, B) and pulsatile (C, D) conditions. Images A and C are control (no-flow), B and D are experimental (flow). Probes fluoresce in the red wavelength; cell nuclei fluoresce green. Images were taken at 10× magnification.

Photoshop, total number of red pixels (intensity >156 on a scale of 1 to 256) were counted in the intimal regions and divided by the total length (in pixels) of the internal elastic lamina. Pixel count was converted to area by the formula $A = cP^2$, where c is the pixel count and P is a one-dimensional pixel length in microns, computed by the Bio-Rad LaserSharp software for the given objective and image capture conditions. Under the image capture conditions used, the optical section thickness was larger than the tissue section thickness.

Twenty-one experiments were performed on porcine carotid arteries ($\bar{P} = 100$: 4 steady, 3 pulsatile, 6 oscillatory; $\bar{P} = 200$: 4 steady, 4 pulsatile) with each experiment generating flow and no-flow control specimens. Two sections of each specimen were stained and two images of each section were taken. Quantitation was performed on all images and averaged for each specimen. Statistical analyses were performed using SAS statistical software (SAS Institute Inc., Cary NC). One observation under high mean pressure, pulsatile flow was discarded as an outlier, three standard deviations away from (and higher than) the mean. To analyze the control, no-flow data, a two-way analysis of variance (ANOVA) was performed to test the hypotheses that pressure and waveform had significant effects. The assumption of normality failed for the both the flow data and the flow data adjusted for baseline by subtracting the paired no-flow control data. A natural log transformation successfully normalized both sets of data; these transformed data were then analyzed with two-way ANOVA to re-test the hypotheses that pressure and waveform had significant effects. The simple effects of pressure on the steady (no-flow and transformed flow) data were also tested. To permit a comparison of control, no-flow values to the paired experimental, flow values, both data sets were transformed with the natural log and tested for the effect of flow (presence or absence).

One unfixed, unfrozen tissue specimen was prepared for scanning electron microscopy (SEM) to observe the details of particle deposition on the intimal surface. This specimen was fixed in paraformaldehyde and stored in Millonig's phosphate buffer at 4°C immediately after *ex vivo* perfusion. Two 2 mm^2 pieces were then cut and rinsed in fresh Millonig's phosphate buffer, post-fixed in 1% osmium tetroxide for 45 minutes at 4°C, dehydrated through graded ethanol solutions, critical point dried, and sputter coated for four minutes with gold/palladium. These specimens were viewed on a JEOL T300 Scanning Electron Microscope.

Results

Qualitative confocal laser microscopy image analysis revealed that spheres were excluded from the media and adventitia for all conditions, in nearly all specimens, and that the most spheres were evident along the intimal surface under oscillatory waveform conditions. Representative microscopy images of particle deposition under steady and pulsatile flow conditions are shown in Fig. 3.

Analyses of the quantified particle deposition data for all pressure and flow perturbations revealed that the waveform was significant ($p < 0.0001$) for the no-flow control data but pressure was not ($p = 0.53$). The interaction between waveform and flow was not significant ($p = 0.56$). For the experimental flow data groups, waveform was significant ($p < 0.001$) but again pressure was not ($p = 0.09$) and no significant interaction was found ($p = 0.35$). To further explore the effect of waveform independent of mean pressure level for the steady and pulsatile conditions, the control (no-flow) values were subtracted from the corresponding experimental (flow) values for each vessel pair to determine the additive increase in intimal deposition with flow at each condition. When the data was adjusted for baseline values in this way (and then transformed to satisfy the assumption of normality), no factors or interactions were significant (all $p > 0.1$). Testing for the simple effect of pressure on the steady groups yielded no significant differences between either the no-flow control ($p = 0.132$) or transformed flow ($p = 0.601$) data. The effect of flow (presence or absence) with all conditions grouped was highly significant ($p < 0.0001$).

The raw data, area of deposited spheres per unit intimal surface

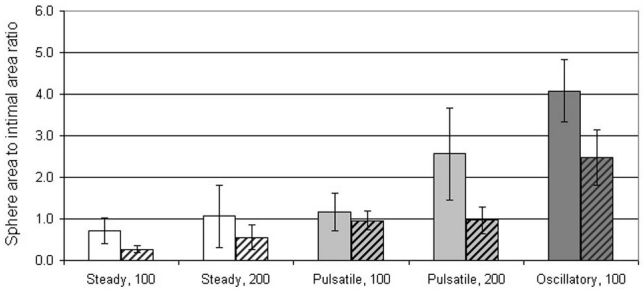

Fig. 4 Sphere area to intimal surface area ratio quantified from conofocal laser microscopy images of vessels perfused under steady (white), pulsatile (light gray) and oscillatory (dark gray) waveforms at mean pressures of 100 mmHg and 200 mmHg. Hatching differentiates flow (unhatched) from no flow (hatched). Bars represent mean±standard deviation.

area, for all conditions are provided in graphical (Fig. 4) and tabular (Table 1) form. The baseline-adjusted data are plotted in Fig. 5.

Scanning electron microscopy revealed spheres clustered and possibly adhered to the intimal surface of a vessel exposed to oscillatory flow conditions (Fig. 6). The non-smooth state of the intimal surface is most likely due to the zero-pressure fixation process and may be a site of some damage to the intima. Histological observations of the tissue revealed endothelial cell coverage in most but not all areas of tissue sections (data not shown).

Discussion

The exclusion of 200 nm particles from the media and adventitia of arteries found in this study agrees well with previous studies. While small particles such as albumin (2 nm) and LDL (20 nm) have been shown to penetrate the intima of perfused arteries [14,15], adenoviral particles (approx. 100 nm) are generally excluded [16]. However, carboxylate-modified polystyrene beads

Table 1 Mean and standard deviation of intimal deposition of particles (area ratio) for all waveform, pressure and flow conditions.

	Waveform:	Steady	Pulsatile	Oscillatory
Flow:	*Pressure:*			
Absent	*100 mm Hg*	0.27±0.08	0.96±0.23	2.47±0.66
	200 mm Hg	0.56±0.30	0.97±0.32	
Present	*100 mm Hg*	0.70±0.31	1.18±0.45	4.08±0.75
	200 mm Hg	1.07±0.75	2.57±1.11	

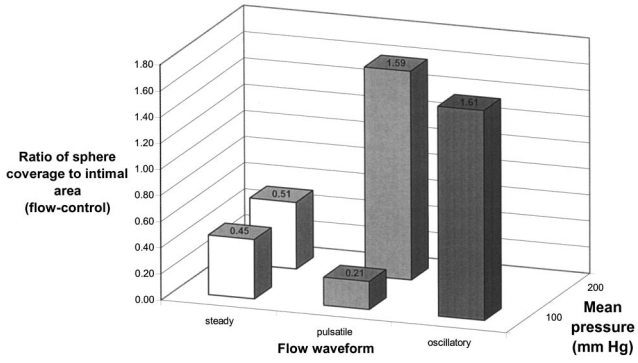

Fig. 5 Baseline-adjusted sphere deposition data for steady, pulsatile and oscillatory waveforms to determine the additive effect of flow. Mean values for each group are shown.

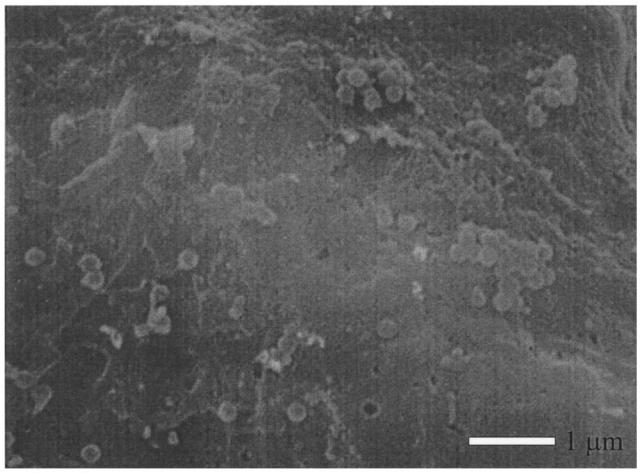

Fig. 6 Scanning electron micrograph showing microspheres clustered and deposited along the intima.

identical to those used here, except 100 nm in diameter, have been shown to reach the media and adventitia of human saphenous veins in low concentrations [17]. The complete exclusion of 200 nm particles not only points toward an obvious size-dependence of intimal permeability but also suggests that the intimal barrier of these vessels was sufficiently intact for the short (8-hour) duration of these experiments to prevent penetration of particles to the media.

Assuming that the intima is completely impermeable to 200 nm particles but somewhat permeable to fluid, the rate of fluid flux per unit length can be estimated from the data obtained in this study. Note that the fluid flux could be driven transmurally by either pressure or concentration gradients. By mass conservation, if the luminal concentration of particles is c, the steady transmural flow Q, the vessel radius and length R and L, respectively, and particle thickness accumulated in time t along the intima x (for $x \gg$ the particle radius), then

$$x = \frac{cQt}{2\pi RL} \tag{1}$$

Thus if changes in radius with time are small, and the concentration of and duration of exposure to particles is consistent, then the intimal deposition of large particles is proportional to transmural fluid flow per unit length. While this relationship may be quite useful under steady flow and no-flow conditions, care must be taken when different waveform conditions are applied. If the transient nature of deposition is different under different waveform conditions, this approximate relationship may not hold.

Nevertheless, transmural vascular fluid flux, estimated with this relationship, may be a clinically relevant quantity. Plasma proteins not wholly excluded from the vessel wall by an intact intima could be driven sub-endothelial by fluid convective forces, which would increase with increasing transmural flow. In particular, small plasma proteins such as LDL may accumulate at atherogenesis-prone sites *in vivo* due in part to locally-increased fluid flow, independent from LDL receptor-dependent mechanisms.

A priori, we had hypothesized (1) that increasing pressure would increase particle deposition, and (2) that flow (presence versus absence) and waveform (steady versus pulsatile versus oscillatory) would affect deposition as well. In particular, we hypothesized that the oscillatory waveform would lead to the most uptake of particles through an increase in permeability. While oscillatory flow did lead to the largest accumulation of particles along the intima, the absence of significant pressure-dependent accumulation is difficult to interpret.

According to Darcy's Law for a homogeneous, incompressible, porous medium with constant permeability k, transmural fluid flow, Q, should be linearly proportional to the transmural pressure gradient:

$$Q = -k \frac{A}{\mu} \frac{dP}{dr} \tag{2}$$

where A is the cross-sectional area of the tissue and μ is the viscosity of the perfusate. We predicted increasing pressure would increase transmural fluid flux and thereby increase intimal particle deposition as particles were excluded from the media by the intima. Contrary to this hypothesis, surface particle deposition did not significantly change with pressure for either the steady or the pulsatile waveform, under either no-flow or flow conditions. In a previous study comparing venous to arterial transport of plasma proteins *in vivo* (such that mean pressures are significantly different), flux of small plasma proteins (<20 nm) showed a marked nonlinear dependence on transmural pressure [15]. Similarly, in human saphenous vein, intimal deposition of adenovirus-sized particles (100 nm) [17] and oligodeoxynucleotides (<5 nm) [18] increased with increasing pressure *ex vivo*. The absence of a significant pressure effect in our data may be due to the small sample size in each group and correspondingly large variances. Alternatively, permeability of the arterial wall, k, may decrease slightly with pressure due to radial tissue compression and compaction, so that increases in transmural fluid flow and thus accumulation of large particles are small (and not significant) as pressure increases. However, this is speculation.

The significance of the waveform variable for the no-flow data indicates that a time-varying pressure profile of physiologically reasonable amplitude increases particle deposition over a nearly steady pressure profile at the same mean pressure. As shown in Fig. 2B, the steady flow perfusion system did generate some pressure fluctuations (residual roller effects), but these were significantly smaller in amplitude than the pulsatile pressure fluctuations. While the mechanism for the increased deposition with the pulsatile and oscillatory waveforms may be increased transmural flow, changes in available intimal surface area cannot be neglected. That is, pressure-induced vessel dilations may have allowed more particles to contact the wall surface during peaks in pressure. However, in the absence of a constant, small transmural flow entraining particles to the vessel surface, it is not clear how the particles would adhere such that they were not released as the pressure decreased. The spheres themselves are designed (carboxylate-modified) to limit non-specific binding. In addition, the suspension solution for particles during perfusion was serum-free. Sphere deposition along the intima should not be receptor-mediated in the absence of protein adherent to the particle surface.

The significance of the waveform variable for the flow data, coupled with the absence of any significance differences in the baseline-adjusted data (Fig. 5), suggests that the presence of axial flow in the range tested does not affect the fundamental mechanisms driving particles to the intimal surface. Since the baseline-adjusted flow data essentially removes the effect of pressure, an effect of pressure was not expected for the steady and pulsatile experiments. Indeed, if we assume, as justified above, that particle deposition is an estimate of transmural flow and then apply Darcy's Law to the baseline-adjusted data, it would appear that permeability is relatively constant with pressure for a steady waveform. In contrast, the pulsatile perfusion results are more complex: at the lower pressure condition the relative permeability is lower than at the corresponding steady condition, but at the higher pressure condition it is much higher. Furthermore, the relative permeability appears to increase with pressure even though these data have been pressure-adjusted by the no-flow data. We can observe that the increase in particle deposition under high pressure pulsatile perfusion (with flow) is several-fold higher than the no-flow deposition, and this difference between pulsatile flow and no-flow is much larger than the difference under steady conditions. Interestingly, the difference between flow and no-flow deposition is of similar magnitude in the high pressure pulsatile and normal (100 mm Hg) pressure oscillatory perfusion conditions. In contrast, under normal mean pressure pulsatile perfusion, the difference between the flow and no-flow deposition is much less than for any other condition, although this result is not statistically significant in the transformed data set. This latter observation argues against a purely pulsation-driven mechanism for increased deposition.

The significance of flow as a variable implicates shear stress as an important factor for particle accumulation. However, experimental artifact may account for some of the difference. As described above, the control vessels had zero mean flow (small flow perturbations occurred with pressure fluctuations but were less than 5 ml/min in amplitude). To equilibrate luminal media pH, oxygenation, and particle concentration with the circulating perfusion media, perfusate in the control vessels was refreshed once each hour from the circulating perfusate. If media had been refreshed twice as often, the difference between flow and no-flow might have been smaller and less significant.

No attempt was made in this study to prohibit radial or circumferential strain as in references [14,18,19] and others. While this manipulation is useful for understanding the role of solid wall deformation in vessel permeability, we were interested in the effects of fluid flow and waveform perturbations on intimal deposition; two pressure levels were tested to assess the pressure-dependence of these flow-mediated effects. Also, a comprehensive assessment of tissue viability and cell-specific functionality post-experiment was not performed. However, all interpretations of the data in this study are comparative in nature. While the absolute values of particle deposition may not reflect *in vivo* values, differences in these values with waveform and flow should hold. We also note that intimal injury, when present, was not so severe that these large particles penetrated into the vessel media.

The steady waveform data permit interpretation with Darcy's Law if we assume the permeability of the arterial wall decreases with pressure due to tissue compression and compaction. While the pulsatile and oscillatory perfusion data are intriguing, they are not particularly amenable to interpretation with Darcy's Law. The assumptions of constant tissue permeability and, more importantly, constant radius and cross-sectional area clearly break down. The mechanisms driving deposition and transmural fluid flux with either time-varying pressure alone or time-varying pressure with axial flow may depend on time-dependent tissue deformations which were not controlled for here. Future work will explore analytical models that do explain and predict particle deposition under these conditions, and explore the quantitative relationship between the intimal accumulation of large particles and transmural fluid flow to better estimate pressure- and flow-dependent tissue permeability in this *ex vivo* system.

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References

[1] Ku, D., 1997, "Blood Flow in Arteries," Annu. Rev. Fluid Mech., **29**, pp. 399–434.

[2] Delfino, A., et al., 1998, "Wall stresses in the carotid bifurcation: effects of wall non-homogeneity and correlation with intimal thickness," J Vasc Invest, **4**(2), pp. 61–71.

[3] Ku, D. N., et al., 1985, "Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress," Arteriosclerosis (Dallas), **5**(3), pp. 293–302.

[4] Stary, H. C., et al., 1994, ''A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association,'' Circulation, **89**(5), pp. 2462–2478.
[5] Frangos, S. G., Gahtan, V., and Sumpio, B., 1999, ''Localization of atherosclerosis: role of hemodynamics,'' Arch. Surg. (Chicago), **134**(10), pp. 1142–1149.
[6] Sill, H. W., et al., 1995, ''Shear stress increases hydraulic conductivity of cultured endothelial monolayers,'' Am. J. Physiol., **268**(2 Pt 2), pp. H535–43.
[7] Jo, H., et al., 1991, ''Endothelial albumin permeability is shear dependent, time dependent, and reversible,'' Am. J. Physiol., **260**(6 Pt 2), pp. H1992–6.
[8] Berceli, S. A., et al., 1990, ''Hemodynamics and low density lipoprotein metabolism. Rates of low density lipoprotein incorporation and degradation along medial and lateral walls of the rabbit aorto-iliac bifurcation,'' Arteriosclerosis (Dallas), **10**(5), pp. 686–694.
[9] Zhao, S. Z., et al., 2000, ''Blood flow and vessel mechanics in a physiologically realistic model of a human carotid arterial bifurcation,'' J. Biomech., **33**(8), pp. 975–984.
[10] Guretzki, H. J., et al., 1994, ''Atherogenic levels of low density lipoprotein alter the permeability and composition of the endothelial barrier,'' Atherosclerosis, **107**(1), pp. 15–24.
[11] Cotran, R., Kutran, V., and Robbins, S., 1989, *Robbins Pathologic Basis of Disease*, 4th ed., W. B. Saunders Co., Philadelphia, PA.
[12] Chesler, N. C., et al., 1998, ''Simplified ex vivo artery culture techniques for porcine arteries,'' J Vasc Invest, **4**, pp. 123–127.
[13] Chesler, N. C., Ku, D. N., and Galis, Z. S., 1999, ''Transmural pressure induces matrix metalloproteinase and matrix degrading activity in porcine arteries ex vivo,'' Am. J. Physiol., **277**, (Heart Circ Physiol 46): pp. H2002–H2009.
[14] Meyer, G., Merval, R., and Tedgui, A., 1996, ''Effects of pressure-induced stretch and convection on low-density lipoprotein and albumin uptake in the rabbit aortic wall,'' Circ. Res., **79**(3), pp. 532–540.
[15] Lever, M. J., and Jay, M. T., 1993, ''Convective and diffusive transport of plasma proteins across the walls of large blood vessels,'' Front Med. Biol. Eng., **5**(1), pp. 45–50.
[16] Rome, J. J., et al., 1994, ''Anatomic barriers influence the distribution of in vivo gene transfer into the arterial wall,'' Arterioscler. Thromb., **14**, pp. 148–161.
[17] MacLennan, M., et al., 2000, ''Pressure increases particle uptaken in human saphenous vein,'' *Advances in Bioengineering*, BED-Vol 48, ASME, New York, pp. 177–178.
[18] Mann, M. J., et al., 1999, ''Pressure-mediated oligonucleotide transfection of rat and human cardiovascular tissues,'' Proc. Natl. Acad. Sci. U.S.A., **96**(11), pp. 6411–6416.
[19] Lever, M. J., Jay, M. T., and Coleman, P. J., 1996, ''Plasma protein entry and retention in the vascular wall: possible factors in atherogenesis,'' Can. J. Physiol. Pharmacol., **74**(7), pp. 818–23.