

USE OF COMPUTATIONAL FLOW MODELING TO CHARACTERIZE THE
BIOLOGICAL RESPONSE OF THE ARTERIAL WALL TO HEMODYNAMICS *IN*
VIVO

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It is generally accepted that hemodynamic shear stresses play a role in the initiation and localization of atherosclerosis, and very likely influence other aspects of the natural history of the disease as well. Much of what we understand regarding the mechanisms by which shear forces affect arteries is derived from *in vitro* experiments that expose monolayers of vascular cells, usually endothelium, to well defined, simple fluid dynamic environments (e.g., 1,2). Though we have learned much from such experiments, this setting is highly unrealistic, and it can be argued that hypotheses derived from studies of cultured cells under simple flows must be tested in experiments where the flow field and the tissue on which it acts are more lifelike.

Naturally, the *in vivo* setting is the most realistic. *In vivo*, vascular cells are on their natural substrate in the entire living organ, are exposed to the natural humoral and hematologic environments, and experience physiologic fluid dynamic and pressure induced stresses and deformations. Only *in vivo* do arteries develop the pathology that motivates this area of vascular research. To establish with confidence the relation between hemodynamics and the developing pathobiology of arterial disease, it is necessary to know in detail the hemodynamic environment in susceptible and atheroprotected segments, in health and in disease

Computational fluid dynamics (CFD) in three-dimensional geometries derived from real vessels can be used to characterize the fluid dynamic environment of the wall in *in vivo* experiments. We can use this tool to examine the biological response to hemodynamic stress by calculating the *in vivo* distributions of hemodynamic variables and relating them to *in vivo* or post mortem biological measurements. This approach can be used for both observational and experimental (i.e., interventional) studies.

We are using this combined approach to characterize, in anesthetized swine, the vascular response to normal hemodynamic stresses and acute changes in vascular flow. Our initial response variable was permeability, measured by injecting the animals intravenously with an albumin-Evans blue dye (EBD) mixture ninety minutes prior to sacrifice. EBD enters the wall bound to albumin and is retained subendothelially; thus the accumulation of the dye in the vessel during the exposure period is a measure of the local albumin permeability of the endothelial interface. Dye accumulation is measured en face as optical density (OD), using photographic densitometry. The spatial variation of the hemodynamic stresses in each animal, at baseline and after flow manipulation, is

obtained from CFD calculations in computational regions whose contours are derived from post-mortem silicone casts of the vascular segments of interest. In this way, we obtain registered spatial maps of both the hemodynamic and response variables in each animal, which can be analyzed statistically.

In our initial application of this technique (3), albumin permeability was correlated against time-average shear stress magnitude ($|\tau_w|$) and oscillatory shear index (4) in three normal swine. The oscillatory shear index (OSI) is a measure of the oscillatory character of the flow and can be related (3) to the residence time of particles near the wall. The region of interest was the proximal portion of the external iliac arteries, extending from the aortic trifurcation to just distal to the circumflex iliac ostium. This region was selected because of its complex geometry, which gives rise to considerable hemodynamic variability. Using local landmarks, a template of the endothelial surface of this region, consisting of about 5000 pixels, was created, to which both the shear and permeability distributions were registered.

This procedure was repeated for each of the six external iliac arteries. For each artery, the pixels were divided into three subgroups according to their shear exposure (lowest, midrange, highest), and the OD values in each vessel were normalized by the average OD of that segment; then, for each subgroup in each artery, the average shear and the average normalized OD (OD_{norm}) of the pixels in that subgroup were computed. The data could be fit by a power law model, $OD_{norm} = 1.39 |\tau_w|^{-0.118}$ ($p < 0.0002$, $R^2 = 0.48$). The permeability exhibited a weaker dependence on the OSI (exponent = 0.032, $p < 0.002$, $R^2 = 0.33$)

Experiments such as these yield a wealth of data. The in vivo hemodynamic environment varies spatially as a matter of course, and that variation is known – quantitatively – from the computations. As a consequence, the effect of variations in hemodynamic variables on the tissue response can be studied in a single animal, reducing the confounding effect of individual variability. It is a bit of an overstatement to say that each pixel of tissue is a separate experiment, since the responses of neighboring pixels are not independent, but with the complete coverage of the tissue afforded by CFD, the range of hemodynamic variables examined in a single experiment is considerable; for instance, the time-average shear stress magnitude in the six arteries described here ranged from 0.3 to nearly 200 dynes/cm².

Looking beyond permeability, we are using this combined approach to seek responses to in vivo hemodynamics at the cellular and molecular levels. By fixing the tissue in situ using protocols optimized for the intended assays, it is possible to preserve the arterial surface and image the morphology of the vascular lining, and the distribution of cytoskeletal and junctional proteins in the endothelium, even after casting. Variations in these structural features along the vessel can then be related to local fluid mechanical stresses. We have used Western blots to quantitate proteins over larger regions pooled on the basis of the hemodynamic calculations. Arterial casting scours unfixed endothelium, so alternative approaches using statistical techniques and noninvasive imaging are being evaluated as means to characterize the hemodynamic environment without casting,

thereby permitting assays on genomic material using quantitative PCR and gene expression arrays. It is also noteworthy that the paradigm presented here has recently been extended to the clinical domain; Stone et al (5) used (steady) CFD calculations in computational geometries derived from clinical intracoronary ultrasound and angiography to relate the remodeling behavior of stented and native arteries to local shear stress exposure.

Most cardiovascular fluid mechanics computations still focus on arterial flows, but mention must be made of the use of CFD to better understand intracellular and pericellular fluid mechanics, and the forces that effect mechanotransduction in vascular endothelium and smooth muscle. Multiscale fluid dynamic computations provide the critical link between conventional vascular hemodynamic variables and the local stresses they generate at the cellular level. The synergism between computational flow modeling and in vivo investigations can be expected to grow in the future as both computational technology and biomolecular science experience new developments, and as the practitioners of each become increasingly aware of the research opportunities afforded by the other.

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