Prediction of Mechanical Properties of Human Atherosclerotic Tissue by High-Frequency Intravascular Ultrasound Imaging
An In Vitro Study

Richard T. Lee, S. Geoffrey Richardson, Howard M. Loree, Alan J. Grodzinsky, Sina A. Gharib, Frederick J. Schoen, and Natesa Pandian

Intravascular ultrasound may be useful for studying the natural history of atherosclerotic lesions of different morphologies and for guiding interventional strategies. This study was designed to test the hypothesis that tissue appearance by intravascular ultrasound is related to the biomechanical properties of atheroma components. Forty-three atheroma caps were obtained from the abdominal aortas of 22 patients at autopsy and studied with an ultra-sensitive, servo-controlled spectrometer. By measuring the static strain caused by increasing levels of compressive stress from 30 to 90 mm Hg, the uniaxial unconfined compression stiffness (ratio of stress to strain) was determined. After mechanical testing, specimens were imaged with a 6F, 20-MHz intravascular ultrasound transducer, and images were interpreted by an investigator who was unaware of the mechanical measurements. Specimens were classified as nonfibrous (n=14), fibrous (n=18), or calcified (n=11) based on intravascular ultrasound appearance. The static stiffnesses of the nonfibrous, fibrous, and calcified ultrasound classes were 41.2±18.8 kPa, 81.7±33.2 kPa, and 354.5±245.4 kPa, respectively (p=0.0002 by analysis of variance). The times to reach static equilibrium (creep time) for the nonfibrous, fibrous, and calcified classes were 79.6±26.5 minutes, 50.2±20.0 minutes, and 19.4±8.1 minutes, respectively (p=0.0007). Intravascular ultrasound appearance was most significantly related to biomechanical behavior when calcium deposits were noted; the differences in biomechanical behavior between nonfibrous and fibrous tissue appearances were less apparent. Important biomechanical behavior of human atherosclerotic tissue can be predicted by intravascular ultrasound imaging; this technology may allow a detailed in vivo assessment of the stress-strain relation in diseased human arteries. (Arteriosclerosis and Thrombosis 1992;12:1-5)

Atherosclerotic lesions are often structurally complex, with varying amounts of lipid, fibrous tissue, and calcium deposits. These components have different biomechanical behaviors that may explain why some plaques are more likely than others to rupture and cause occlusive thrombosis.1 Coronary angiography does not provide information about plaque structure beneath the endothelial surface (with the exception of severe calcification), so that evaluating the clinical importance of plaque structure antemortem has been limited to characterizing lumen geometry.2 Ultrasonic tissue-characterization methods can differentiate various pathological patterns of atherosclerosis,3 and these patterns dramatically influence the biomechanical behavior of human plaque compo-
Mechanical Testing was further increased to 90 mm Hg (-12.0 kPa), and was bathed in room-temperature normal saline and were obtained; and in four patients, three specimens were obtained. Abdominal aortic plaques were se-


ted to provide large and flat specimens most amenable to accurate uniaxial mechanical testing. Plaques were selected when they were at least 5 mm from a vessel ostium; visibly uncomplicated, with no surface fracture or overlying thrombus; and at least 9 mm in diameter. The fibrous cap was dissected free of adventitia, media, necrotic plaque components, and any adjacent normal aortic tissue.

Methods

Specimens

Forty-three fibrous caps from human atheroscle-

rotic plaques were harvested from the abdominal aortas of 22 patients during routine autopsies at Brigham and Women’s Hospital and the Beth Israel Hospital, Boston, Mass. In five patients, one specimen was obtained; in 13 patients, two specimens were obtained; and in four patients, three specimens were obtained. Abdominal aortic plaques were selected to provide large and flat specimens most amenable to accurate uniaxial mechanical testing. Plaques were selected when they were at least 5 mm from a vessel ostium; visibly uncomplicated, with no surface fracture or overlying thrombus; and at least 9 mm in diameter. The fibrous cap was dissected free of adventitia, media, necrotic plaque components, and any adjacent normal aortic tissue.

Mechanical Testing

Mechanical testing was performed with an ultrasen-
sitive, servo-controlled mechanical spectrometer (Dyn- nastat, Imass Inc., Hingham, Mass.).4 The fibrous cap was bathed in room-temperature normal saline and held between an acrylic base that was connected to an actuator mechanism and a 7-mm-diameter cylindrical stainless steel platen that was connected to the spectrometer load cell (Figure 1). An initial uniaxial compres-
sive stress equivalent to 30 mm Hg (-4.0 kPa) was ap-
plied normal to the surface of the plaque. Creep was allowed to continue until static equilibrium was achieved; for this study, equilibrium was defined as the point in time when, during the previous 5 minutes, the increase in strain was less than 2% of the total strain caused by the imposed stress. The thickness of the specimen after the initial 30 mm Hg stress was mea-
ured with the spectrometer; the mean thickness was 1.0±0.4 mm. After equilibrium was obtained, the stress was further increased to 90 mm Hg (-12.0 kPa), and creep was again allowed to continue until equilibrium; the data reported in this study correspond to the strains and time periods (“creep times”) produced by this second stress. From the stress and strain data, the uniaxial unconfined compression stiffness was calcu-
lated as the ratio of increase in equilibrium stress to strain. After mechanical testing, a portion of the spec-
imen was fixed in 10% neutral buffered formalin for histological studies.

Intravascular Ultrasound Imaging Studies

Twenty-six specimens were immersed in normal saline immediately after mechanical testing and im-
aged within 24 hours. For 17 additional specimens, plaques were preserved in 10% neutral buffered formalin and imaged at a later date for logistic reasons. Specimens were suspended by sutures in a normal saline bath and imaged with a commercially available intravascular ultrasound probe (Sonicateh, Boston Scientific Corp., Watertown, Mass.). This device consists of a 6F disposable catheter enclosing a mechanically rotated driveshaft with a 20-MHz ultrasound crystal at its tip. The best axial resolution is 0.3 mm, and lateral resolution is 0.5 mm. The catheter is used in conjunction with an imaging console adapted for operation at 20 MHz and 360° scans (Diasonics, Milpitas, Calif.). The specimens were imaged at a constant distance from the catheter tip. First, a series of images of the specimens was obtained with a constant gain setting, and later the gain was intentionally reduced to the lowest level to assess the degree of ultrasound reflectivity at the lowest gain setting. The images were stored on videotape and interpreted by an investigator who was unaware of mechanical measurements or the gross appearance of the tissue. Plaques were qualitatively characterized by an a priori classification as predominately nonfibrous, fibrous, or calcified, based on their appearance by intravascular ultrasound (Figure 2). When specimens demonstrated uniform low-intensity echo reflections throughout the specimen, they were classified as nonfibrous. When at least 30% of the specimen area was composed of higher-intensity echo reflections at the preselected constant gain setting but demonstrated marked attenuation in echo
reflectivity with low gain settings, they were classified as fibrous. When at least 10% of the specimen area had a high degree of echo reflectance typical of dystrophic calcification and retained considerable echo reflectivity even at the lowest gain setting, the specimen was classified as calcified.

Histological Studies
Specimens for histology were embedded in paraffin, cut in cross section at 5–6 μm, and stained with hematoxylin and eosin. Specimens were classified as cellular, hypocellular, or calcified in the manner previously described by a pathologist who had no knowledge of the results of mechanical testing or ultrasound imaging. Cellular specimens consisted of smooth muscle or other cells in a connective tissue matrix. When cells were sparse, specimens were classified as hypocellular. Calcified specimens had abundant granular calcific deposits.

Statistical Analysis
The data were analyzed by analysis of variance, with patient identification included as a random effect to account for multiple samples from single patients. The effect of storage in formalin or imaging immediately in normal saline was included as a fixed effect. The classification by intravascular ultrasound imaging and the interaction between ultrasound appearance and whether the specimen was imaged in saline or formalin were included as fixed effects. The static stiffness was the dependent variable in the first analysis; in the second analysis, the time to equilibrium (creep time) was the dependent variable. Post hoc testing was performed with Tukey’s studentized range statistic. The relation of intravascular ultrasound class to histological class was tested with the $\chi^2$ test. In all testing a value of $p<0.05$ was considered to be statistically significant; results are presented as mean±SD.

Results
Intravascular Ultrasound Imaging
Of the 43 specimens tested, 14 were classified as nonfibrous by intravascular ultrasound appearance, 18 were classified as fibrous, and 11 were classified as calcified. There was a significant correlation of histological class with intravascular ultrasound class ($\chi^2=32.8, p<0.0001$). All 11 specimens classified as calcified by ultrasound were also classified as calcified by histological examination. Of the 18 specimens classified as fibrous by ultrasound, the majority ($n=11$) were classified as hypocellular, four as cellular, and three as calcified on the basis of histological examination. Of the 14 specimens classified as nonfibrous by intravascular ultrasound, six were classified as cellular and eight as hypocellular by histological examination.

Mechanical Testing
For specimens classified as nonfibrous by intravascular ultrasound, the creep strain was 23.5±10.5%, and the static stiffness was 41.2±18.8 kPa. For fibrous specimens, the strain was 11.4±4.5%, and the static stiffness was 81.7±33.2 kPa; for calcified specimens, the strain was 3.1±1.7%, and the static stiffness was 354.5±245.4 kPa (Figure 3). Intravascular ultrasound imaging class was significantly related to static stiffness ($F=9.21, p=0.0002$). The effects of whether the specimen was imaged in formalin or saline and the random effect of patient identification were not significant; in addition, the interaction of whether the specimen was imaged in formalin or saline with intravascular ultrasound class was not significant. Post hoc testing demonstrated a significant difference in static stiffness between specimens classified by ultrasound as calcified versus those classified as nonfibrous and those classified as fibrous ($p<0.05$), but the difference in static stiffness between nonfibrous and fibrous was not significant.

For specimens classified as nonfibrous by intravascular ultrasound, the creep time was 79.6±26.5 minutes; for fibrous specimens, the creep time was 50.2±20.0 minutes; for calcified specimens, the creep time was 19.4±8.1 minutes (Figure 4). Intravascular ultrasound imaging class was significantly related to creep time ($F=11.38, p=0.0007$). Whether the specimen was imaged in formalin or saline and the random effect of patient identification were not
**Discussion**

Although insight into the mechanical properties of atherosclerotic plaque components will be a prerequisite for understanding plaque rupture, relatively little is known about these properties. One reason that atherosclerotic plaque biomechanics have not been extensively explored is that methods to define internal plaque structure in vivo have not been available. Angiography provides prognostic clues about probability of future infarction, but many lesions that lead to future myocardial infarction appear mild by angiography. In this study, plaque appearance by intravascular ultrasound was related to two parameters of biomechanical behavior. The static stiffness was significantly related to ultrasound class, although the difference between the two “softest” classes (nonfibrous and fibrous) was not statistically significant. The creep time, a parameter reflecting the viscoelasticity of the tissues, was significantly different between each of the three classes. Although the stress applied in this study was below angioplasty stresses, these data suggest that intravascular ultrasound imaging could be useful in guiding percutaneous angioplasty; currently, selecting the magnitude of balloon pressurization and the duration of inflation is based on operator experience.

This study indicates that intravascular ultrasound technology may be adapted to perform detailed structural analysis of atherosclerotic vessels in vivo. Using the technique of finite-element computer analysis, Richardson et al found that structural analysis based on histological cross sections of coronary lesions accurately predicts plaque rupture points. With miniaturization and improved resolution of intravascular ultrasound equipment, finite-element structural analysis based on images obtained at catheterization may soon be available. This technique has the potential of identifying lesions that are not angiographically severe but are structurally unstable, with points of high stress concentration. Understanding plaque stability may provide insight into the mechanism of plaque fracture, the major cause of acute vascular syndromes. Several mechanisms of acute mechanical injury to plaque have been proposed; possibly, more than one mechanism is responsible.

**Limitations**

There are several limitations in this study. First, and possibly most important, the parameters defined in this study do not completely describe the complex mechanical behavior of the plaque. The definition of static equilibrium used in this study was empirical; static stiffness would not have changed significantly with a different definition, although creep times would be different. In addition, the stress–strain relation of biological materials is rarely linear. Although this study addressed the increase in strain that resulted from an increase in stress near the range of physiological blood pressure, the stiffness of these materials would be higher under the pressures imposed by angioplasty; whether specimens from each ultrasound class show proportionally increased stiffness at higher stresses was not explored in this study. The minimum number of parameters to describe the behavior of a perfectly elastic, isotropic material is two (i.e., Young's modulus and Poisson's ratio). However, biological materials are rarely iso-
tropic,15 and the creep data in this study demonstrate that plaque components are not perfectly elastic. The nonelastic nature of these specimens is also apparent when considering dynamic stiffness described in our previous study with the same experimental apparatus and a separate set of specimens; dynamic stiffness values at frequencies near physiological heart rates are approximately one order of magnitude greater than static stiffness values referenced to comparable initial equilibrium stress.

A second potential limitation concerns the "idealized" in vitro conditions used to image specimens in this study. In vivo imaging will be more subject to changing gain settings and other technical factors that may limit the ability of ultrasound to predict mechanical behavior. It is possible that the use of quantitative integrated backscatter methods would be superior to the qualitative classification used in this study.3 In addition, even when specimens appeared grossly homogeneous at necropsy, ultrasound imaging demonstrated tissue heterogeneity; thus, the data in this study represent global averages of each specimen. The heterogeneity of plaque components and the use of a priori classifications for histology and ultrasound may explain why ultrasound did not reliably distinguish hypocellular from cellular histological classes. Another potential concern in this study is that 17 specimens were fixed in formalin and imaged later. However, previous studies have demonstrated little effect of fixation on ultrasound tissue appearance,7 and no effect of fixation was found in the analysis used in this study.

To obtain more homogeneous tissues, the necrotic core and more normal vessel components beneath the fibrous cap were dissected away from the specimens used in this study; it is important to recognize the structural interactions of the atheroma cap with these other components. The mechanical properties of the soft, lipid-rich material will significantly influence the distribution of stress in the diseased vessel. Although the precise mechanical behavior of the lipid core will be difficult to define because small changes in temperature or lipid composition change its characteristics,16 the core is much softer and bears very little circumferential stress. For this reason, the precise mechanical properties of the lipid pool will not significantly influence a structural analysis as much as the definition of the lipid pool's size and geometry. With continued technical advances, intravascular ultrasound may be well suited to this task.

Finally, creep is a complex function that reflects both the viscous nature of the solid matrix (viscoelasticity) and the movement of interstitial water (poroelasticity).17 The different creep times measured in this study probably reflect different time-dependent behavior of various types of tissues. More experiments need to be performed to quantitatively separate poroelastic versus viscoelastic contributions to the mechanical behavior of atheroma caps.

Clinical Implications

Intravascular ultrasound may provide a new method of defining plaque structure beneath the endothelial surface that will allow detailed structural analysis of the plaque in the catheterization laboratory. Although much greater insight into the biomechanics of atheroma components will be necessary, accurate antemortem prediction of mechanical behavior of these complex structures may become feasible.

References


Key Words • biomechanics • stiffness • stress
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