Arterial Wall Characteristics Determined by Intravascular Ultrasound Imaging: An in Vitro Study

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The feasibility of assessing arterial wall configuration with an intravascular 40 MHz ultrasound imaging device was investigated in an in vitro study of 11 autopsy specimens of human arteries. The system consists of a single element transducer, rotated with a motor mounted on an 8F catheter tip. Cross sections obtained with ultrasound were matched with the corresponding histologic sections.

The arterial specimens were histologically classified as of the muscular or elastic type.

Muscular arteries interrogated with ultrasound presented with a hypoechoic media, coinciding with the smooth muscle cells. In contrast, the media of an elastic artery densely packed with elastin fibers was as echogenic as the intima and the adventitia. On the basis of the cross-sectional image, it was possible to determine the nature of the atherosclerotic plaque. The location and thickness of the lesion measured from the histologic sections correlated well with the data derived from the corresponding ultrasound images.

This study indicates that characterization of the type of artery and detection of arterial wall disease are possible with use of an intravascular ultrasound imaging technique.

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Intravascular ultrasound has the unique ability to provide cross-sectional images of the arterial wall. First results with mechanically driven single element intravascular ultrasound imaging systems have been reported and show great promise (1–8). This report describes the results of in vitro experiments with an intravascular ultrasound imaging device currently under development in our laboratory.

The objectives of this study were to test the possibility of ultrasound to 1) study the normal arterial wall morphology in autopsy specimens of human arteries; 2) study the ability to determine the nature of the atherosclerotic lesion; 3) measure the extent to which the lesion occupies the vessel circumference; and 4) measure the area of the lumen and compare the results with those obtained from the histologic sections. The results provide a basis for future in vivo applications.

Methods

Human artery specimens. Common carotid and iliac arteries were removed at autopsy from humans (six male, five female; age range 18 to 72 years) within 8 to 19 h after death. In this study, the arteries were stored frozen rather than fixed with formalin as suggested by others (9,10). Initial studies indicated that formalin had a major influence on echographic characteristics, resulting in a twofold increase in echo strength. Because we have found that fresh material does not result in a different echographic response from that of the corresponding thawed segments, our results from thawed segments should be more compatible with the situation in vivo. Arterial specimens approximately 10 mm long were frozen and stored at −20°C. For in vitro studies, the specimens were thawed and embedded in a 1.2% solution of agar-agar. Care was taken to select straight arteries to allow adequate access of the ultrasound catheter in the artery. The lumen of the artery was filled with purified water.

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Intravascular ultrasound system. A 40 MHz single element transducer mounted on an 8F catheter tip was used. Axial resolution of the system was 75 μm, with lateral resolution >200 μm at a depth of 1 mm. Cross-sectional images were obtained by catheter tip rotation. Data acquisition time for one complete image was 20 s. The resulting images were displayed on a monitor by means of a videoscanned memory of 512 × 512 pixels with 256 shades of gray. Marker lines were displayed for calibration purposes.

Ultrasound examination. In vitro experiments were carried out by placing the catheter in the lumen of the artery. With the help of A-mode imaging, optimal positioning in the arterial lumen was achieved so that perpendicular sonification was performed over the length of the artery. Subsequently, marked cross sections were obtained of the artery from proximal to distal, with a 1 mm interval between each.

Microscopic examination. After the ultrasound studies, the proximal site of the arteries was correspondingly marked with ink. The arteries were fixed in 10% buffered formalin for 12 h, and subsequently decalcified in a standard Cal Ex solution (Fisher Inc.) for 5 h. The arteries were processed for routine paraffin embedding. Transverse sections (5 μm thick) perpendicular to the longitudinal axis of the artery were cut at 1 mm intervals. The sections were arranged from proximal to distal. The histologic sections were stained with the Verhoeff's elastin van Gieson stain technique. With the Verhoeff's elastin stain, a highly selective black staining of elastin fibers is obtained. The van Gieson technique is used as counterstaining for muscle and connective tissue (11). In addition, sections were stained with the hematoxylin azophloxine technique to delineate connective tissue and calcium.

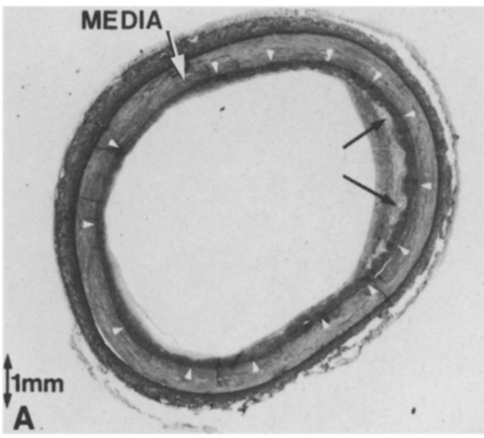
Analysis of data. From each of the 11 artery specimens studied, one echographic image with the most significant plaque thickness was selected and compared with the corresponding histologic section. The arterial wall and plaque characteristics as documented by histologic study were compared with the corresponding echographic cross sections. For quantification purposes, photographs were made of the histologic sections together with a calibrator indicating a 1 mm scale in depth. The slides were projected on paper and manually traced. The lumen and internal elastic lamina were independently traced by two investigators for histologic and ultrasound analysis. The traced boundaries were digitized and analyzed by computer to measure plaque thickness and luminal area. A modified centerline analysis method based on the left ventricular wall motion analysis of Bolson et al. (12) was used to determine the radial plaque thickness between both boundaries at 100 positions. These positions were distributed equally over the arterial wall in a clockwise orientation (Fig. 1).

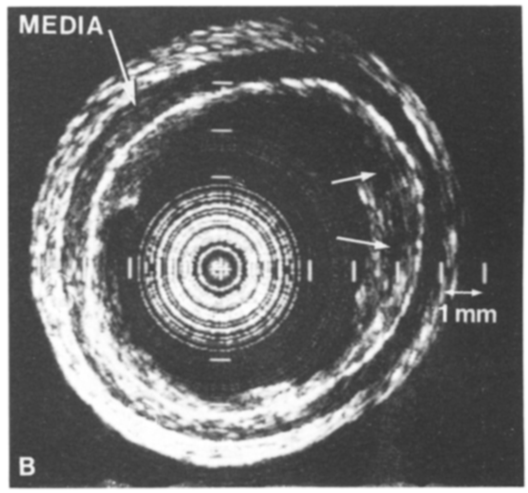
Results

Arterial wall morphology. Microscopic examination showed two types of artery: a muscular artery and an elastic artery. The difference between the two is found in the media. The media of a muscular artery is composed of smooth muscle cells and was practically devoid of elastin fibers (Fig. 1). In contrast, the media of an elastic artery consists mainly of densely packed, concentrically arranged elastin fibers amidst smooth muscle cells (Fig. 2). On ultrasound examination, a muscular artery was recognized by the hypoechoic media, with clear definition of the internal and external elastic lamina showing bright echoes (Fig. 1). An elastic artery was recognized by a media that was as bright as its surrounding tissues (Fig. 2). We hypothesize that the presence of elastin fibers regularly arranged in the media was responsible for a significant amount of acoustic backscatter with a power level comparable with that from the intima and adventia. If this classification (hypoechoic versus bright) is used, intravascular ultrasound imaging can provide a correct distinction between a muscular (iliac [n = 5]) and elastic (carotid [n = 6]) artery in all instances.

Plaque characteristics. Deposits of lipids seen in the histologic section appeared as a hypoechoic area in the lesion on ultrasound examination. A fibromuscular lesion, which is mainly composed of smooth muscle cells and disorderly arranged elastin fibers, was represented by soft echoes. When dense fibrous tissue or calcified deposits were present, bright echoes were seen in the corresponding echographic cross section. The difference between the two is that, in the presence of fibrous tissue, the internal elastic lamina can still be visualized beyond the lesion, whereas calcifications obscure this structure.

In all 11 arteries studied, there was evidence of a lesion. In nine arteries, soft echoes were noted attached to the intima. These represented lesions of a fibromuscular nature (Fig. 1 to 3). Fibromuscular plaques were to a variable degree covered by a dense fibrous cap, which on ultrasound imaging was recognized as a more reflective structure separating the fibromuscular lesion from the arterial lumen (Fig. 3). Five of these nine arteries showed within the plaque a hypoechoic zone that corresponded with the lipid deposits seen on the histologic section (Fig. 1 and 3). In three of the nine arteries, in addition to the fibromuscular proliferation, there were lesions with strong reflections beyond which neither the media nor the adventitia could be seen (Fig. 3). Corresponding histologic examination revealed calcifications amid dense collagen. In the two remaining arteries, a classic atherosclerotic lesion appeared on the ultrasound image, with variable echo amplitudes recognized as hypoechoic soft and bright echoes (Fig. 4). Beyond the lesion, the intima, media and adventitia were barely visible. The plaque was covered with a fibrous cap and composed of lipid, fibromuscular tissue and spots of calcification.





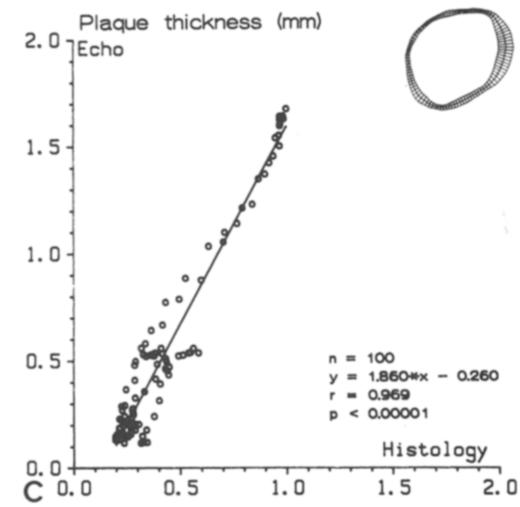


Figure 1. Histologic section (**A**) obtained from a typical iliac artery and a corresponding echographic cross section (**B**). The media, composed of smooth muscle cells, appears hypoechoic. An atherosclerotic lesion of fibromuscular nature is recognized with ultrasound as relatively soft echoes attached to the bright echo surface of the intima (**arrowheads in A**). Lipid deposits present in the histologic section correspond to hypoechoic zone (**arrows**) on the ultrasound study. Plaque thickness (**C**) measured in a clockwise orientation from the histologic section and the ultrasound section is shown in the diagram (**inset**). Note the close relation in the measurements obtained with both techniques. (Verhoeff van Gieson stain; original magnification ×8, reduced by 20%.)

Plaque location and thickness. The luminal boundaries were always distinct, as were the boundaries of the internal elastic lamina on the histologic sections. Echographically, problems were encountered in identifying the internal elastic lamina in the presence of calcification. Because of strong reflection and attenuation, the echoes of this structure (that is, internal elastic lamina) were obscured. Plaque thickness measurements of regions where this phenomenon occurred were excluded for comparison.

Among the 11 arteries studied, a total of 1,100 positions were analyzed to calculate plaque thickness from both the histologic and the corresponding echographic section. In 331 positions, no lesion was present, whereas in 577 positions, plaque thickness could be calculated (Fig. 5). In 192 positions, plaque thickness boundaries of the internal elastic lamina were obscured by the presence of calcium deposits within the lesion. The site and extent of the lesion were accurately determined from the echographic images. Thickness derived from histologic study proved to be lower.

Internal luminal area. There was no distinct correlation between histologic and echographic measurements in the calculation of luminal area (Fig. 5). The reason for this observation might be mechanical deformation during preparation and cutting of the histologic sections (see also Fig. 2). The luminal area as determined by histologic study was usually smaller compared with the data derived from echographic imaging.

Discussion

In this study, the ultrasonic appearance of human arteries in relation to the corresponding histologic sections was investigated. The results might form a basis for future in vivo application.

Distinction between muscular and elastic arteries. Accurate distinction was possible between the different types of arteries studied. Absence of concentrically arranged elastin fibers in the media of a muscular artery may be the main underlying reason why the media appeared hypoechoic (compare Fig. 1 and 2). This observation is not in accordance with that of Meyer et al. (7), who suggested that "the muscular media appeared hypoechoic due to the relative lack of collagen." In our opinion, this discrepancy could be explained by their omission to perform an elastin stain

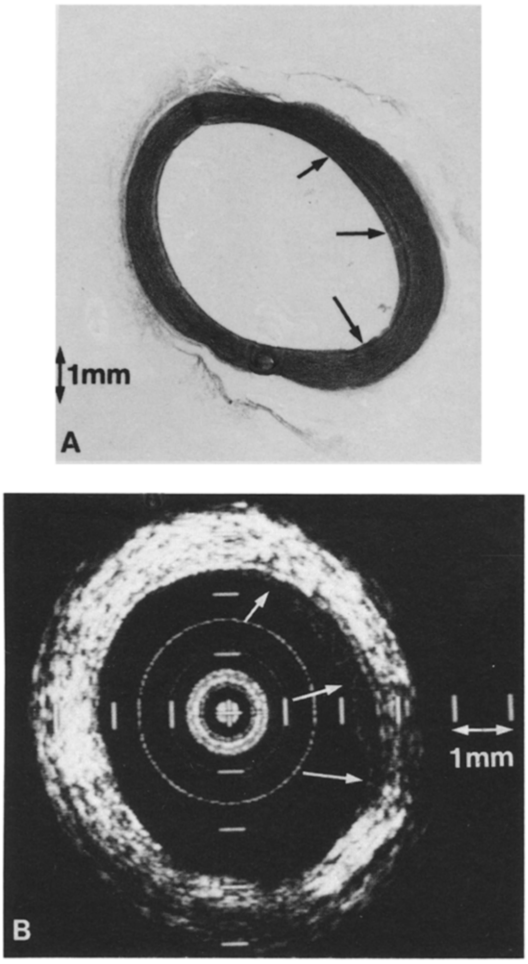


Figure 2. Histologic cross section (**A**) obtained from a typical carotid artery and a corresponding echographic section (**B**). The media of an elastic artery, mainly composed of concentrically arranged elastin fibers, appears to be as bright as the intima and adventitia (compare with Fig. 1). A fibromuscular lesion (<0.5 mm thick) is found to be restricted to the 12 and 4 o'clock positions (**arrows**). Note that the histologic section (**A**) appears oval-shaped as a result of mechanical manipulation. (Verhoeff van Gieson stain; original magnification ×8, reduced by 10%.)

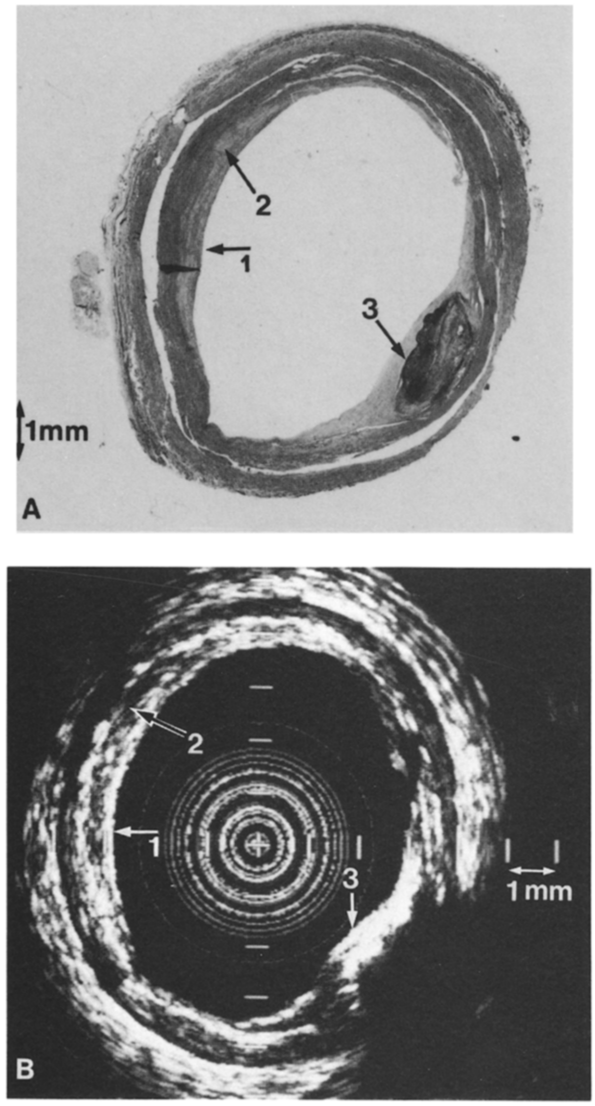


Figure 3. Histologic section (**A**) and corresponding echographic cross section (**B**) obtained from an iliac artery with an advanced atherosclerotic lesion extending along the entire circumference of the vessel. The lesion was predominantly of fibromuscular nature, characterized by soft echoes attached to the intimal surface. Microscopically, it was found that the boundaries of this plaque presented with dense organized fibrous tissue. Echographically, this structure appears with relative bright echoes (**arrow 1**). Microscopically, the noted lipid deposits on the histologic section at the 11 o'clock position correspond to the hypoechoic zone on the ultrasound study (**arrow 2**). At the 4 o'clock position, a distinct atherosclerotic lesion is seen, characterized by bright echoes (**arrow 3**). Because of attenuation and reflection of the ultrasound caused by calcification in this lesion, no echoes were derived from the intima, media or adventitia. (Hematoxylin azophloxin stain; original magnification ×8, reduced by 10%.)

procedure like the Verhoeff's elastin technique (11). Moreover, they believed that collagen was the single component responsible for ultrasonic backscatter (13,14).

We assume that, with in vivo studies, atherosclerotic lesions might be more readily envisioned in a muscular artery presenting with an hypoechoic media rather than in an elastic artery. In the elastic artery, the echographic characteristics of intima, media and adventitia are comparable. To identify an additional lesion attached to the intimal surface, the lesion needs to be composed of tissue with distinctly different echographic characteristics. This statement is supported by the work of Pignoli et al. (9), who indicated that wall measurements were less accurate when additional echoes were present within the media.

Influence of lesion composition on ultrasound characteristics. An interesting observation arising from this study was that using a high frequency transducer, sound transmission was appreciably influenced by the composition of the lesion.

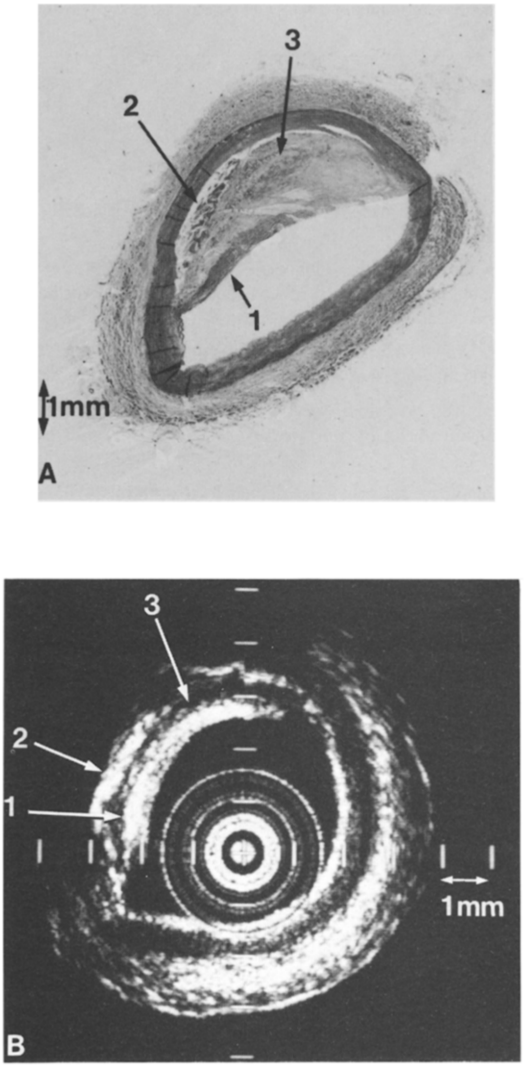


Figure 4. Comparison between histologic cross section **(A)** and corresponding echographic section **(B)** from an iliac artery with an advanced atherosclerotic lesion. **A,** A classic atherosclerotic lesion is seen between the 8 and 2 o'clock positions. The fibrous cap is bright **(arrow 1)**; thereafter fibromuscular tissue is visible, corresponding to soft echoes. A second bright echo structure **(arrow 2)** beyond which neither the media nor adventitia is visualized, corresponds to calcification on the histologic section. Lipid deposits present as a hypoechoic zone **(arrow 3)**. (Verhoeff van Gieson stain; original magnification ×8, reduced by 10%.)

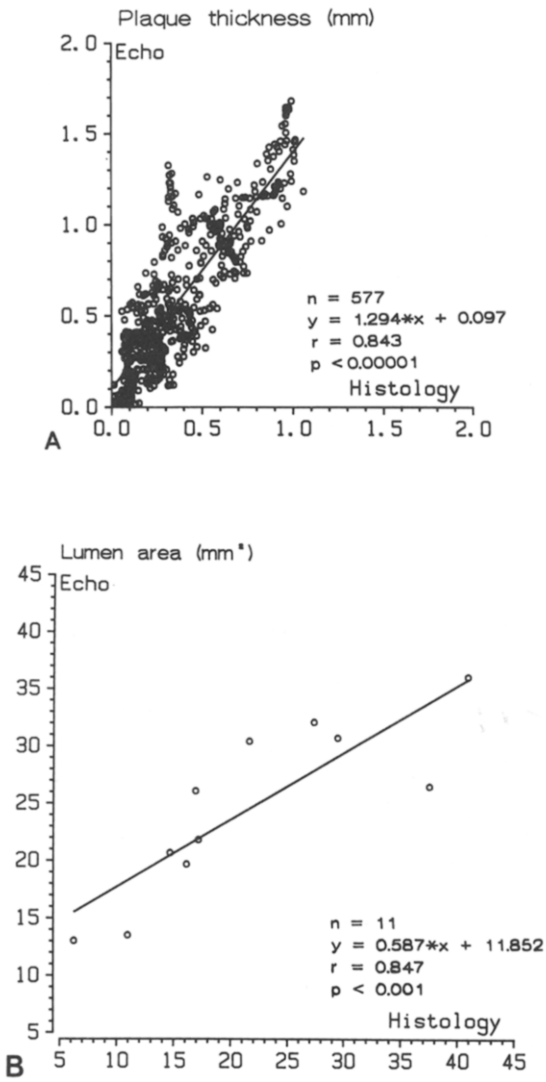


Figure 5. Comparison of plaque thickness **(A)** (577 sections) and luminal area **(B)** (11 arteries) evaluated by histology and intravascular ultrasound. Note that the luminal area as determined by histology was usually smaller, probably because of shrinkage after dehydration during fixation.

Lipid deposits were hypoechoic. Fibromuscular lesions were imaged as soft echoes attached to the intimal surface. It should be noted that a fibromuscular lesion consists mainly of connective tissue, smooth muscle cells and elastin fibers. The elastin fibers, however, are arranged in disorderly fashion in contrast to the elastin fibers in the media of the elastic arteries. It is probable that the net result of backscatter is intimately related to the arrangement of the elastin fibers.

Dense fibrous tissue that contained a significant amount of collagen presented as bright echoes. This observation is in agreement with that of O'Donnel et al. (14). Bright reflections, however, were also seen in the presence of calcium deposits. Because echo amplitudes are also a function of distance from the transducer (because of acoustic field properties and attenuation), the diagnosis of calcification was made not from the echo strength, but from the presence of shadowing behind the structure. It must be stressed that the current study was obtained without blood. Technical evolution to date indicates that, in the near future, in vivo application may not be hampered by blood.

Plaque thickness measurements. In this study, plaque thickness measurements derived from histologic examination were generally lower than those derived from the corresponding echographic sections. This was most likely a result of the shrinkage after dehydration during fixation (10,15). Although the histologic cross sections were very similar to the corresponding echographic sections, the data derived from luminal area measurements did not correlate well. Because luminal area is extremely dependent on the shape of the artery, we suspect that mechanical deformation during the preparation of the histologic sections was responsible for this discrepancy. These problems are avoided when anatomic measurements are obtained from gross examination (8). In addition, phantom studies have shown that the performance of the present ultrasound system can accurately determine the internal vessel diameter.

Possible clinical applications. The current image quality in vitro shows that in the early stage of development, atherosclerotic lesions may be detected in vessels showing no reduction in luminal size due to dilation of the arterial wall (16). Development and evolution of the atherosclerotic process on the basis of plaque composition and extension along the arterial wall circumference are a potential clinical application. Phantom studies revealed that the dynamic area of the artery interrogated might be adequately determined. This implies that one potential application of intravascular ultrasound imaging is its use before and after percutaneous catheter balloon dilation. With this method, the effect of dilation can be assessed, not only on the net increase in the luminal area, but also on the structural changes in the plaque and arterial wall.

Accurate determination of the location and composition of the atherosclerotic lesion with intravascular ultrasound imaging seems to be another important application. One of the main problems of intravascular tissue removal techniques is damage of normal wall tissues, which includes the risk of perforation. Ultrasonographic control enables the operator to remove just a reasonable amount of the lesion. Moreover, determination of the suitability for angioplasty based on the echographic definition of the plaque composition could be another major application.

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