Tissue Characterization of Arterial Wall Based on Elasticity Imaging with Ultrasound

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Abstract: This paper describes a noninvasive method for evaluating regional elasticity of atheroma by measuring the minute change in thickness of the arterial wall during one heartbeat. By comparing the pathological findings with the elasticity images, elasticity distributions for lipid and fibrous tissue (mixture of smooth muscle and collagen fiber) were determined in vitro. Furthermore, to characterize the fibrous cap of plaque, which almost consists of smooth muscle and collagen fiber, the correlation between the collagen content and elasticity was investigated for fibrous tissue. Based on these elasticity reference data, fibrous tissue surrounding the soft inclusion of lipids was noninvasively clarified in a carotid plaque and the collagen content in the fibrous cap was also estimated. This method offers potential for detection of plaque vulnerability in a clinical setting.

Introduction

Rupture of atherosclerotic plaque is probably the most important factor underlying the sudden onset of the acute coronary syndrome [1]. Direct characterization of the composition and vulnerability of atherosclerotic plaque, rather than of the angiographic lumen [2], may offer insight into the mechanisms of plaque regression and progression [3] and thereby promote evaluation of cholesterol-lowering therapy [4] for reduction of cardiovascular events. MRI and intravascular ultrasound are promising technologies for directly imaging plaque morphology [5]. For the evaluation of dynamic mechanics, arterial elasticity has been determined by measuring the rough change in the diameter of the artery [6]. However, a method to detect the vulnerability of atherosclerotic plaque with sufficient accuracy has not yet been reported. The purpose of this study was to measure cross-sectional elasticity images in arterial walls with transcutaneous ultrasound.

Materials and Methods

In Vitro Experimental Setup: Figure 1 shows the experimental setup for in vitro experiments using excised human femoral arteries. The change in internal pressure was generated using an artificial heart, and the internal pressure was measured with a pressure catheter (Camino 110-4) placed in the lumen of the artery. The artery fixed in the water tank was measured with a linear probe (center frequency: 7.5 MHz).

Figure 1: Experimental setup.

Elasticity Estimation: [7,8] An ultrasonic beam was sequentially scanned at M (=60) positions with a linear-type ultrasonic probe of 7.5 MHz using conventional ultrasound diagnostic equipment (Toshiba SSH-140A), and multiple (N_m+1) points were preset from the luminal surface to the adventitia along the m-th ultrasonic beam (m=1,...,M) with constant intervals of \( h_0 = 375 \mu \text{m} \) at a time \( t_0 \) just before the ejection period. By dividing the arterial wall into multiple layers, we defined the n-th layer (n=1,...,N_m) as being between two contiguous points, n and n+1, along each beam. For measurement of change in thickness of each of the N_m layers, the instantaneous depth \( x_{n,m}(t) \) of the n-th point along the m-th beam was simultaneously tracked by applying the phased tracking method to the received ultrasound. The minute decrease of several tens of micrometers in wall thickness of the n-th layer resulting from the arrival of the pressure wave at the beginning of the ejection period was determined by \( \Delta h_{n,m}(t) = x_{n,m}(t) - x_{n,m}(t) \).

In the phased tracking method, for calculation of the auto-correlation function between the quadrature demodulated signals of sequentially received echoes, minute phase change of about 0.4 degrees caused by movement of the n-th point during the pulse transmission interval \( \Delta T \approx 200 \mu \text{s} \) can be accurately determined by introducing a constraint, namely, that...
their waveforms are identical but their phase values change. The lowest value of the change in thickness was validated as being about 0.5 μm by expanding a rubber plate in a water tank. Such a minute change in thickness cannot be measured by any other method. From the ratio of the maximum decrease in thickness during one heartbeat, \( \Delta h_{\text{max}} \), to the initial thickness \( h_0 \), the maximum deformation of the \( n \)-th layer was determined by \( \Delta E_{n,m} = \Delta h_{\text{max}}/h_0 \). Since the deformation was sufficiently small and was in the linear regime, it showed incremental strain in the radial direction. By assuming that the arterial wall is incompressible and that the blood pressure is applied normal to each layer, the elastic modulus of the \( n \)-th layer along the \( m \)-th beam, \( E_{n,m} \), is approximately given by \[ E_{n,m} = \frac{h_0}{\rho_n \cdot h_0 \cdot N_m} \frac{N_n - n + 1}{N_n} \frac{\Delta p}{(n=1,\ldots,N_m; m=1,\ldots,M)} \] where \( \rho_{n,0} \) is the initial inner radius of the curvature of the \( n \)-th layer along the \( m \)-th beam at a time \( t_0 \). We assumed that the pressure in the arterial wall decreases linearly with the distance from the intimal side to the adventitia and that the arterial wall is almost isotropic.

For the region with a length of 18 mm along the axis of the artery, the regional elasticity \( E_{n,m} \) was estimated on the cross-sectional image. Since the reflected ultrasound was received at a sampling interval of 100 ns (=75 μm along depth direction) after the quadrature demodulation, we further divided each layer with a thickness of \( h_0 \) into 5 points, shifted the initial depth of each layer by 1/5 of \( h_0 \) and applied the above procedure to each depth. Thus, \( E_{n,m} \) was estimated at intervals of 75 μm in the depth direction and 300 μm in the axial direction. Using a silicone rubber tube with two layers set in an artificial circulation system, the accuracy of the measurement of regional elasticity for each layer has already been validated to be about 0.1 MPa, that is, the error is about 8% of the elasticity value obtained by a separate static pressure-diameter test.

Construction of Elasticity Distribution:[8] In this study, the elasticity distributions were obtained with respect to fibrous tissue (mixture of smooth muscle and collagen) and lipids. After ultrasonic elasticity measurements, the arteries were fixed in formalin. The plane scanned by ultrasound was identified by imaging a needle, which was fixed in the external surface of the posterior wall, in the B-mode image during the ultrasonic measurement. The pathological images of the measured planes were made with elastica-masson staining. By referring to the pathological images, the regions which correspond to fibrous tissue and lipids were assigned in the corresponding cross-sectional elasticity images. From the regions assigned for respective tissues, elasticity distributions were determined.

Analysis of Correlation Between Collagen Content and Elasticity:[10] Fibrous tissue consists mainly of collagen fibers and smooth muscle. The correlation between the collagen content and the elasticity was investigated with respect to fibrous tissue to noninvasively estimate the collagen and smooth muscle contents based on the measured elasticity. In the elasticity image, there are \( M \) ultrasonic beams and \( N_m \) regions along \( m \)-th beam \((m=1,\ldots,M)\). Therefore, in an elasticity image, there are \( N_m \) regions with respective elasticity values. Each region in an elasticity image is referred as \( R_{n,m}^c \) \((m=1,\ldots,M; n=1,\ldots,N_m)\).

As shown in Fig. 2, each region \( R_{n,m}^c \) in the elasticity image is compared with the corresponding region \( R_{n,m}^p \) in the pathological image assigned as follows: The entire pathological image, which corresponds to the entire elasticity image, is divided into \( M \) sections in the axial direction of the artery, and each \( m \)-th section of \( M \) sections is divided into \( N_m \) regions in the radial direction. The sizes of all regions in an elasticity image are equivalent. However, actual sizes of regions assigned in the pathological image differed because of the distortion of the arterial wall due to dehydration during formalin fixation. In this study, by assuming that the change in size due to distortion is homogeneous, each \( M \) section was divided into \( N_m \) regions of equivalent size in the radial and axial directions. Then, the collagen content of each region \( R_{n,m}^c \) assigned in the pathological image was estimated using the Mahalanobis distance. In this study, the composition of each region \( R_{n,m}^c \) is classified into one of four classes \((i=1:\text{collagen}, 2:\text{smooth muscle}, 3:\text{elastin}, 4:\text{background})\). The mean, \( \mu = (\mu_x, \mu_y, \mu_z)^T \), and covariance matrix, \( \Sigma \), of RGB values were determined for each class \( i \) by manually assigning typical regions in the pathological image for each class. From the determined mean \( \mu \) and the covariance matrix \( \Sigma \), the Mahalanobis distance \( d_i^2 \) between each pixel, \( X = (x_r, x_g, x_b) \), in the region \( R_{n,m}^c \) and the center of the \( i \)-th class is obtained as follows:

\[ d_i^2 = (X - \mu_i)^T \Sigma^{-1} (X - \mu_i). \]
deformations unavoidably occur. Such deformations lead to differences in size and shape between the elasticity and pathological images. Therefore, we used a part of the arterial wall showing little differences in size and shape between the elasticity and the pathological images.

The resolution was too small to exactly correlate a region in the elasticity image to the corresponding region in the pathological image because of the deformation caused by dehydration. Therefore, we employed spatially averaged values obtained from regions of 0.6 mm in the axial direction and 0.6 mm in the radial direction in size.

**Results**

Figure 3(1) shows two typical elasticity images of iliac arteries measured *in vitro*. Figure 3(2) shows pathological images of the corresponding sections. By referring to pathological images, lipids and fibrous tissue (mixture of smooth muscle and collagen) were determined as shown in Fig. 3(2), and the corresponding regions were assigned in elasticity images as shown in Fig. 3(1). Figure 3(3) show elasticity distributions of respective assigned regions. By applying the same procedure to nine arteries, mean and standard deviation in elasticity were determined to be 81±40 kPa and 1.0±0.63 MPa for lipids and fibrous tissue, respectively (Fig. 3(4)).

**Collagen Content vs. Elasticity:** Pathological images shown in Fig. 4(b) were divided into small regions which correspond to the resolution of elasticity images (Fig. 4(a)). Figure 5 shows the relationship between the collagen content and elasticity. Plots and vertical bars show spatial mean and standard deviation in each 600 μm×600 μm region. Positive correlation was found in Fig. 5 and the regression line was estimated by the least-squares method. Figure 6 shows the relationship between smooth muscle and collagen contents in each small region in pathological images, and fibrous tissue is found to almost consist of smooth muscle and collagen. Therefore, both collagen and smooth muscle contents in fibrous tissue are estimated using the regression line in Fig. 5.

**In Vivo Tissue Characterization:** Based on the elasticity reference data determined by *in vitro* experiments, an elasticity image (Fig. 7(a)), which was noninvasively measured *in vivo*, was classified as one of three categories (lipid, fibrous tissue, or other). In Fig. 7(b), regions classified as lipids and fibrous tissue were colored by yellow and cyan, respectively. Furthermore, smooth muscle and collagen contents in fibrous tissue were estimated as shown in Fig. 7(c).
Figure 6: Relationship between smooth muscle and collagen contents in each small region in pathological images shown in Fig. 4(b).

Conclusions

Cross-sectional images of the elasticity around atherosclerotic plaque were transcutaneously measured in this study. By comparing the elasticity and pathological images, elasticity distributions of lipids and fibrous tissue were determined as the ‘elasticity library’. Using the elasticity library, the elasticity image measured with transcutaneous ultrasound was classified into lipids and fibrous tissue. Furthermore, a positive correlation was found in the relationship between elasticity and the collagen content in fibrous tissue, and this results offers a potential for estimating collagen content in the classified fibrous tissue. The proposed novel approach has a potential for non-invasive tissue characterization and diagnosis of the vulnerability of plaque in a clinical setting.

References


Figure 7: (a) Elasticity image of a carotid plaque of 71-year-old male with hyperlipidemia. (b) Detected fibrous tissue and lipids. (c) Estimated smooth muscle and collagen contents of fibrous cap.