Mapping elasticity moduli of atherosclerotic plaque \textit{in situ} via atomic force microscopy

Philippe Tracqui, Alexis Broisat, Jackub Toczek, Nicolas Mesnier, Jacques Ohayon, Laurent Riou

\textsuperscript{a}Laboratoire TIMC-IMAG, Equipe DynaCell, CNRS UMR 5525, Institut de l’Ingénierie et de l’Information de Santé, Faculté de Médecine de Grenoble, 38706 La Tronche Cedex, France
\textsuperscript{b}INSERM, U877, Radiopharmaceutiques Biocliniques, Faculté de Médecine de Grenoble, 38706 La Tronche Cedex, France

\textbf{A R T I C L E   I N F O}

Article history:
Received 2 September 2010
Received in revised form 20 December 2010
Accepted 24 January 2011
Available online 4 February 2011

Keywords:
Atherosclerosis
ApoE\textsuperscript{−/−} mouse
Force-spectroscopy
Stiffness mapping
Vulnerable plaque

\textbf{A B S T R A C T}

Several studies have suggested that evolving mechanical stresses and strains drive atherosclerotic plaque development and vulnerability. Especially, stress distribution in the plaque fibrous capsule is an important determinant for the risk of vulnerable plaque rupture. Knowledge of the stiffness of atherosclerotic plaque components is therefore of critical importance. In this work, force mapping experiments using atomic force microscopy (AFM) were conducted in apolipoprotein E-deficient (ApoE\textsuperscript{−/−}) mouse, which represents the most widely used experimental model for studying mechanisms underlying the development of atherosclerotic lesions. To obtain the elastic material properties of fibrous caps and lipidic cores of atherosclerotic plaques, serial cross-sections of aortic arch lesions were probed at different sites. Atherosclerotic plaque sub-structures were subdivided into cellular fibrotic, hypocellular fibrotic and lipidic rich areas according to histological staining. Hertz’s contact mechanics were used to determine elasticity (Young’s) moduli that were related to the underlying histological plaque structure. Cellular fibrotic regions exhibit a mean Young modulus of 10.4 ± 5.7 kPa. Hypocellular fibrous caps were almost six-times stiffer, with average modulus value of 59.4 ± 47.4 kPa, locally rising up to ~250 kPa. Lipid rich areas exhibit a rather large range of Young’s moduli, with average value of 5.5 ± 3.5 kPa. Such precise quantification of plaque stiffness heterogeneity will allow investigators to have prospectively a better monitoring of atherosclerotic disease evolution, including arterial wall remodeling and plaque rupture, in response to mechanical constraints imposed by vascular shear stress and blood pressure.

\textcopyright \ 2011 Elsevier Inc. All rights reserved.

1. Introduction

Recent experimental studies clearly showed that the mechanical properties of the cellular microenvironment, notably its rigidity or stiffness, play a critical regulatory role for a variety of fundamental cell behaviors and responses (Janmey et al., 2009). This is especially true in diseases such as atherosclerosis, where inflammatory, biological and mechanical processes act in concert to remodel the arterial wall structure and composition (Arroyo and Iruela-Arispe, 2010; Hallow et al., 2009). Remodeling of the arterial wall intima, media and adventitia layers could ultimately lead to the formation of a plaque that may become vulnerable, i.e. prone to rupture (Finn et al., 2010). Rupture of coronary atherosclerotic plaques and subsequent myocardial infarction are the most frequent fatal consequences of coronary artery disease, which accounts for more than 50% of cardiovascular deaths (Lloyd-Jones et al., 2010). Among determinants for vulnerable plaque rupture, concentration of mechanical stress in the plaque fibrous capsule is recognized as a major risk factor, since this area becomes prone to rupture beyond a critical stress amplitude threshold. Due to atherosclerotic plaque heterogeneity, this peak stress value highly depends on the respective stiffness of atherosclerotic plaque components (Ohayon et al., 2008). Therefore, knowledge of plaque components stiffness becomes of critical importance to precisely assess stress values and location within the plaque, and thus to evaluate the risk of plaque rupture.

However, accurate measurements of the mechanical properties of atherosclerotic plaques are still lacking. This is an especially challenging task since plaque development is responsible for the formation of very heterogeneous regions, including a lipidic and necrotic core surrounded by a fibrous cap. Indeed, atherosclerotic plaque formation involves a cascade of inflammatory processes originating with mononuclear cells adhering to the endothelial surface and evolving to a mass of fibrous layers capping lipid-filled macrophages (Finn et al., 2010).

The aim of this study was to quantify the highly heterogeneous mechanical characteristics of atherosclerotic plaques developing in the mouse aortic arch over a 30-week time period. Specifically, we used the apolipoprotein E-deficient (ApoE\textsuperscript{−/−}) mouse, the most widely used experimental model for studying the development of

\textsuperscript{*}Corresponding author. Fax: +33 4 56 52 00 22.
E-mail address: philippe.tracqui@imag.fr (P. Tracqui).

1047-8477/ – see front matter \textcopyright \ 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2011.01.010
atherosclerotic lesions since it spontaneously develops atherosclerotic lesions with morphology similar to those observed in humans. In this murine model, feeding animals with a high-fat diet accelerates lesion development. Typically, plaque formation originates in the aortic root and progresses to the ascending aorta, the aortic arch and onward through the aorta’s principal branches.

In this present work, we made use of atomic force microscopy (AFM) to generate high spatial resolution AFM data relevant to the mechanical characterization of atherosclerotic lesions. As a surface probe method for local elasticity measurements of biological samples, AFM has been rather extensively used to examine mechanical properties of different types of living cells and of sub-cellular entities (Francis et al., 2010; Mahaffy et al., 2004; Radmacher, 2007). However, its use as a micromanipulation tool for studying macroscopic tissue properties is far less extended and mostly restricted to bone and cartilage tissues (Stolz et al., 2004; Tao et al., 1992) or extracellular matrices (Darling et al., 2010; Loparic et al., 2010; Soofi et al., 2009). Using AFM force spectroscopy in combination with specific labeling of cellular, fibrotic and lipidic plaque components, we provided here a detailed spatial mapping of the heterogeneity of atherosclerotic plaques mechanical properties.

2. Material and methods

2.1. Animal model

Five-weeks old female ApoE−/− mice on a C57/BL6 J background (n = 5) were obtained from Charles River Laboratory. The animals were fed a western-type diet containing 20% casein milk and 0.15% cholesterol (Safe).

2.2. Tissue sampling and preparation

ApoE−/− mice were euthanized by an overdose of pentobarbital at the age of 25–30 weeks when atherosclerotic lesions are clearly visible. The aorta was quickly harvested and immersed in cold 4 °C Krebs–Henseleit buffer containing 0.026 mM EDTA (KH). The aortic arch was carefully dissected free from adhesive tissue under a binocular. The aortic arch was embedded in optimal cutting temperature (OCT) medium and frozen in liquid, nitrogen-cooled isopentane. Serial 16 μm-thick and 6–8 μm-thick adjacent transversal slices were obtained for AFM measurements and for histological and immunohistological stainings, respectively (Fig. 1). A hydrophobic pen was used to draw a water-repellent barrier that kept the aortic ring immersed into KH buffer throughout the entire experimental protocol. Pilot studies were conducted using adjacent atherosclerotic wall arterial sections used as fresh sample or submitted to a freeze–thawing step, respectively. Measurements were performed in similar and corresponding regions of the fresh (fs) and freeze–thawing (th) samples, respectively. Both the range of Young’s moduli values and the profile of the histogram of the compiled moduli values can be compared, with typically three clusters of moduli values that can be attributed to different plaque components, namely lipid rich, cell rich and hypocellular regions, as further evidenced in Section 3. Globally, we did not observe a significant influence of the freeze–thawing step on our AFM measurements. This is illustrated by the following data set, obtained from the probing of two adjacent sections that is representative of measurements we carried out with different matching pairs of arterial sections (30-week animal, 116 probed locations, lipid rich area $E = 2.86 ± 2.40$ kPa (fs) and $E = 3.32 ± 2.59$ kPa (th); cell rich area $E = 12.85 ± 1.47$ kPa (fs) $E = 10.97 ± 1.71$ kPa (th); hypocellular area $E = 22.21 ± 4.86$ kPa (fs) $E = 23.25 ± 3.98$ kPa (th), (non significant differences, p > 0.05).

2.3. Histology and immunohistology

The following standard histological stainings were performed: (i) a trichrome, haematoxylin, erythrosine, safran (HES) staining the nucleus, cytoplasm and fibrosis in blue, pink and yellow, respectively, (ii) an oil red-O staining of lipid deposits in red, and (iii) a smooth muscular cells (SMC) staining using an anti-α-actin antibody for the evaluation of SMC proliferation and fibrous cap formation. Following a 1-h blocking step at room temperature (RT) (mouse serum, 10%), the alcalin phosphatase-coupled anti-α-actin antibody (A5691, Sigma, 1:800) was applied for 1 h at RT. Permanent red as the chromogen was applied and the sections were counterstained with haematoxylin.

Histological and immunohistological staining were performed both prior and after AFM measurements, thereby providing for AFM a precise knowledge of plaque composition and structure.

2.4. AFM force spectroscopy testing

Mechanical testing was performed on a total of 200 sites over arterial wall cross-sections with a servo-controlled AFM (Nanowizard II, JPK instruments, Berlin, Germany). Serial 16 μm-thick vessel sections were mounted on Poly-L-Lysine slides (Thermo-Scientific, France). The arterial wall, including the intima and media layers – were exposed for probing by AFM. Tissue samples were kept in KH buffer. All AFM measurements were made in liquid at room temperature, including cantilever calibration (see below). Tissue section thickness was checked by recording the difference between the contact points of force curves on the substrate and on the arterial ring. For all specimens, mechanical testing was completed within 4 h of harvesting the sample.

2.4.1. Samples indentation by AFM

Samples were indented by borosilicate sphere-tipped cantilevers (diameter of 5 and 12 μm Novascan, USA) having a nominal spring constant between 0.1 and 0.3 N/m. Cantilever stiffness was determined from thermal calibration following manufacturer procedure.

To obtain the elastic properties of the investigated area of a sample, the force-spectroscopy mode was employed: force curves were recorded, with the piezo displacement and laser deflection signals continuously fed to a microcomputer system. The conventional Hertz’s model is only applicable to measure elasticity.
in the probed regions, even if extended formulations of the force-indentation relationships have been proposed to analyze viscoelastic properties through frequency-dependent AFM measurements (Mahaffy et al., 2000, 2004). Thus, our characterization of the arterial wall elasticity requires that the force indentation data are dominated by the permanent elasticity of the sample, with the contribution of viscosity being minimized. Several studies reported that such minimization is obtained in the small approach velocity domain (Kang et al., 2008; Hassa et al., 1998), i.e. in quasi-static loading conditions, while at high velocities apparent elastic modulus may increase due to viscous effects (Francius et al., 2006). A prototype model for such mechanical response is the standard linear solid (a spring-dashpot in parallel with another spring), used as a basis for a viscoelastic extension of the Hertz’s model of cell testing by AFM (Darling et al., 2007). In our study, measurements of the elastic modulus of the arterial wall cross-sections, were performed at small approach velocities around \( \frac{0.6}{\text{m/s}} \), for which the elasticity modulus remains independent, within standard deviation, from the piezodrive velocity.

Arterial sections were probed in multiple locations. Line scanning of the arterial wall was conducted according to a predefined grid (force mapping). However, because of the large heterogeneity of tissue stiffness, manual scanning was also conducted, the choice of probing zones being guided by the pre-staining of the samples in order to identify potential areas of interest within the arterial wall (elastic lamina, intra lamina spaces) and within the atherosclerotic plaque (necrotic core, fibrous cap, Fig. 2). Each retained site was probed with typically five force-indentation curves each in order to limit the risk of probe contamination by detached pieces of cells or tissues.

### 2.4.2. Identification of the probed plaque components

Histo- and immunohistological stainings allowed the choice and identification of areas probed by AFM. Indeed, registration of digital pictures from adjacent cross sections used respectively for AFM experiments and for histo- and immunohistological staining was performed using histological landmarks that could be identified on both such as elastic lamellae. This procedure allowed the prospective identification of zones to be probed. Alternatively, the cross sections used for AFM probing were also subsequently stained for histo- and immunohistological characterization, thereby allowing the retrospective and direct identification of AFM-probed areas.

### 2.5. Data analysis of the force curves

It is known that the sample thickness might bias the estimation of the Young’s modulus of the AFM-probed elastic medium (Dimitriadis et al., 2002). Since arterial tissue cross-sections were sufficiently thick (15–20 \( \mu \text{m} \)) with regard to the indentation depth (0.5–1 \( \mu \text{m} \)), the probed section can be considered as a semi-infinite substrate. It is known from the seminal works of Hertz (1882) and Boussinesq (1885) (see Johnson, 1985, for an overview) that the contact force \( F \) exerted on a sphere indenting a linear elastic and semi-infinite medium is proportional to the power 1.5 of indentation depth \( d \) such that:

\[
F(d, R, E, m) = K \frac{d^{3/2}}{d_{0}^{3/2}}
\]

where the contact modulus \( K \) can be written as:

\[
K = \frac{4}{3} \frac{E}{(1 - v^2)}
\]

where \( E \) and \( v \) are the local Young’s modulus and Poisson’s ratio of the elastic material, respectively. In the following, we will assume that arterial wall and plaque components behave as incompressible materials (\( v = 0.5 \)). Thus, one gets for \( F \) the following relationship:

\[
F = \frac{16E}{9} \sqrt{R d^{3/2}}
\]
By applying the fit of the Hertz model to the force-distance curves using $E$ and the contact point as fit parameters (JPK IP fitting software, Berlin, Germany), we identified a Young’s modulus for each force-distance curve. Force-distance curves exhibit an overall very good fit to the Hertzian contact model (Fig. 3), with some curves displaying a softer initial indentation of the material. In these cases, the best fit to the largest portion of the indentation curve, which corresponds to a constant value of the Young’s modulus, was retained to get the elasticity modulus value.

2.5.1. Validation of AFM mechanical testing

Testing of our force-spectroscopy approach was conducted by probing a rubbery synthetic polymer, Polyvinyl Acetate (PVAc). PVAc cylindrical samples (5 mm diameter) with different stiffness were obtained by polymerization of vinyl acetate monomer through 1 or 6 freeze–thawing cycles. Young’s moduli values of 300 μm thickness slices of PVAc samples, considered as incompressible materials, were measured using both a micropipette aspiration technique, according to a procedure previously published by our group (Boudou et al., 2006) and by performing compression tests using a dynamic mechanical testing instrument (GABO Eplexor, Ahlden, Germany, load cell of 25 N, sensor sensitivity of $10^{-4}$ at full range).

With micropipette experiment, Young’s moduli of $23 \pm 7$ kPa and $173 \pm 73$ kPa, were identified for the soft (1 freeze–thawing cycle), and for the stiff (6 freeze–thawing cycles) samples, respectively. Compression tests gave mean values of $17.6 \pm 3.4$ kPa and $145.4 \pm 30.8$ kPa for soft and stiff PVAc samples, respectively (Le Floch et al., 2010).

For AFM testing, the PVAc thin slices were mounted on a cover-glass and glued on their borders. Samples were indented in their middle part by borosilicate sphere-tipped cantilevers (5 μm diameter, 0.17 N/m stiffness). PVAc sections were probed at five different locations with at least three force-indentation curves at each point. Fitting the Hertz’s model against the force-distance curves gives Young’s moduli of $18.9 \pm 7.1$ kPa and of $201.2 \pm 54.6$ kPa for the softer and stiffer PVAc samples, respectively. Standard deviations come mostly from heterogeneity of the samples stiffness, which varies quite significantly from one location to another. Taken all together, the estimation of Young’s moduli obtained by the three methods are quite coherent and thus provides a strong support for our AFM measurements procedure.

![Fig. 3. Measuring the stiffness of atherosclerotic plaque components in ApoE−/− mice by AFM indentation.](image)

(A) Variables involved in the Hertz’s model solution for spherical indentation of an elastic medium with Young’s modulus $E$ and Poisson’s ratio $v$. For an imposed displacement $Dz$ of the cantilever, the indentation depth $d$ depends on the cantilever stiffness $k$. (B) Illustrative force distance curve recorded in our study for deflection $d$ of the cantilever during indentation (approach curve) of hypocellular fibrotic plaque component. The best fit (solid line) of the theoretical force – indentation relationship (Eq. (3)) against deflection values translated into force amplitude (dashed thick line) provides the Young’s modulus value of the probed site. Figure insert provides the corresponding Log–Log plot of the force-indentation data curve that can be compared to the theoretical straight line with slope 1.5 given by the Hertz’s model solution for the fitted Young’s modulus value. The goodness of fit is weaker near the contact point, as discussed in the text.
3. Results

3.1. Characterization of arterial wall by AFM

The arterial wall is a heterogeneous and composite medium that is well described as a multilayer structure made of superimposed, more or less wavy, elastic sheets (Fig. 4A). In between, SMC cells and extracellular matrix proteins are found.

Measurements conducted on artery sections gave a mean elastic lamella thickness of 2.4 ± 0.4 μm, while the inter lamella space was of 9.2 ± 2.0 μm (Fig. 4A). AFM indentation of such structures (Fig. 4B) provided force distance curves that, after data fitting by the Hertz’s model, gave for elastic lamina and inter-lamina space mean Young’s modulus values of 58.3 ± 6.3 kPa and 4.2 ± 0.7 kPa, respectively.

3.2. Characterization of plaque components by AFM

According to the atherosclerotic lesion labeling determined by HES, oil red O and SMC stainings, we subdivided each plaque into three distinct regions (Fig. 5):

- An hypocellular fibrosis area (HyFb area), defined as a hypocellular area with a strong fibrosis staining on trichrome HES images,
- A cell rich fibrosis area (CeFb area), defined as a fibrosis area colonized by smooth muscle cells (SMC) originating from the inner media,
- A lipid rich area (LpRi area), defined either as macrophage-derived foam cell-rich area or as an extracellular lipid-rich area and colored in red following oil red O staining.

Stiffness mapping of thinly sectioned atherosclerotic arteries was analyzed globally from co-localization of elasticity measurements throughout the tested regions and examination of the corresponding areas given by staining. Thanks to our registration procedure (Fig. 6), a precise identification of the probed site could be made. For any given plaque, significant variations exist in the identified elasticity modulus. Fig. 7 shows a line scan performed on the fibrous and cap region, with indication of the different staining for lipids, cells and extra-cellular matrix proteins.

The mean values and range of variation we obtained for the elasticity moduli within each region are summarized in Table 1 for the five plaques that were analyzed. Globally, 200 sites were probed, either as line scans across regions of interest, or independently to test specific structures.

As shown in Table 1, the Young’s modulus of the cellular fibrosis areas (E_{CeFb} = 10.4 ± 5.7 kPa) is roughly two times larger than the elastic modulus derived for lipid rich areas (E_{LpRi} = 5.5 ± 3.5 kPa). The variability of the elastic moduli of the hypocellular fibrosis regions is rather large, with histogram of identified Young’s modulus values exhibiting a bimodal distribution (Fig. 8), with a mean value E_{HyFb} = 59.4 ± 47.4 kPa. This variability reflects the heterogeneity of the histological sections, evidenced by the different stainings that have been used. Indeed, mechanical properties of fibrotic plaque regions are expected to highly depend on the respective ratios of cells, lipids and ECM proteins.

The above classification of elastic modulus values are based on histological discrimination of the plaque regions as cellular or hypocellular fibrosis. One can notice that an overlapping exists between the ranges of values obtained for these two different fibrotic regions. Thus, we checked if the mean values we obtained would
be significantly affected by imposing a threshold Young's modulus value as a discriminative boundary between cellular and hypocellular fibrosis mechanical properties. We re-computed the Young's modulus mean values by taking the first quartile \( Q_1 \) of the set of hypocellular fibrosis moduli values, i.e. 21.8 kPa (Table 1), as such a discriminating threshold value. Then, the mean values for hypocellular fibrosis only slightly increases at \( E_{HyFb} = 67.4 \pm 46.6 \) kPa, while the recomputed value for cellular fibrosis is \( E_{CeFb} = 10.6 \pm 5.4 \) kPa. Thus, separating criterion based either on histological recognition or upper bound elasticity values provides coherent and similar characterization of plaque stiffness heterogeneity.

4. Discussion

Although multiple techniques have been used to measure the mechanical properties of healthy and diseased arterial walls (Engler et al., 2004; Loree et al., 1994a; Matsumoto et al., 2004), few studies tried to quantify the mechanical properties of plaque components (Barrett et al., 2009; Loree et al., 1994b). Using atomic force microscopy, this is the first study, to our knowledge, that provides in situ stiffness measurements of atherosclerotic plaque components using AFM.

Several difficulties arise when dealing with characterization of plaque components: the first one is the large heterogeneity of the plaque in terms of composition and mechanical properties. Second, using AFM heads on inverted microscopes is rather challenging when using thick tissue samples since the bottom sample surface is imaged while the upper surface is probed. Thus, a main challenge is the ability to link rather precisely the obtained elasticity (Young's) moduli to the underlying histological structure of the plaque.

The approach we proposed here overcomes these issues and provides a direct correlation between the in situ quantification of plaque components stiffness and the corresponding probed histological structure. Using micrometer-sized spherical indenter tips gives rise to macroscopic elastic properties of the probed plaque.
components. If considering plaque heterogeneity, this provides a good compromise between accuracy and averaging of elastic moduli quantification of tissue structures characterized by their content in cells, lipids and proteins. Of importance, similar Young’s moduli were obtained from measurements conducted with spherical tips of different sizes, which lends additional confidence to the values reported here.

Our AFM-based measurements of arterial wall elasticity agree with reported values obtained in other studies and for similar or distinct experimental models, as discussed below. Upon removal of cells, collagen fibers and other ECM components, the isolated elastin network along the bovine thoracic aorta, when tested under equi-biaxial tensile tests, exhibit nonlinear and anisotropic mechanical response. The elastin network has an initial tangent modulus of about 30 kPa in the longitudinal direction, while it is roughly ten times stiffer in the circumferential direction (Zou and Zhang, 2009). Considering that elastic fibers are made primarily of the protein elastin, the former value agrees rather well with the value we reported here for elastic lamina. The elasticity moduli we found are also in agreement with the ones recently reported by Oie et al. (2009) for healthy small-caliber porcine artery. Using a tactile mapping system with a probe of diameter 1 μm, the Young’s modulus values they measured for elastin-rich regions are 50.8 ± 13.8 and 69.0 ± 12.8 kPa in the media and in the lamina elastica interna, respectively, while elasticity modulus decreases to 17.0 ± 9.0 kPa in other regions in the media (Oie et al., 2009).

Engler et al. (2004) reported elastic moduli in the range 5–8 kPa for the medial layers of carotid arteries excised from 6-month-old pigs, i.e. with a lower bound value close to the mean Young’s modulus of 4.2 ± 0.7 kPa we reported here in mouse aorta for inter-lamina space stiffness. In contrast, Matsumoto et al. (2004) reported quite higher values, with elasticity moduli of ~180 kPa for elastic lamina and ~50 kPa for inter-lamina spaces for porcine aortic media. Such differences may be explained by differences in the anatomical nature, histology and anisotropic properties of the different type of arteries, in addition to aging and inter-species variations.

Regarding plaque component stiffness, our results indicate a rather large heterogeneity in the stiffness of the fibrotic layers surrounding the lipidic core of the plaque, as illustrated by the line scan measurements reported (Fig. 7). Typically, the Young’s moduli in cell rich fibrosis (CeFb) regions appear to be 5–6 times lower than those measured in hypocellular fibrosis (HyFb) areas. Lee et al. (1991) reported for human atherosclerotic plaques that hypocellular caps were, on average, approximately 1–2 times stiffer than cellular caps, with dynamic stiffness at 1 Hz of 900 ± 220 kPa versus 510 ± 220 kPa, respectively. This ratio is lower than the one reported here between hypocellular and cellular fibrotic regions, with absolute values also significantly higher in human than in mouse. Later, Lee et al. (1992) analyzed human abdominal aortic plaques, with specimens being classified as non-fibrous and fibrous according to intravascular ultrasound imaging. Using uniaxial creep-compression tests, they reported Young’s modulus values for non-fibrous and fibrous tissue of 41.2 ± 18.8 kPa and 81.7 ± 33.2 kPa. This latter value is quite comparable to the mean value $E_{HyFb} = 59.4 ± 47.4 kPa$ we reported here. For a seek of comparison with other fibrotic tissues, let us notice that Berry

\begin{table}
\centering
\begin{tabular}{lcccc}
\hline
Plaque areas & Young modulus (kPa) & Modulus range (kPa) & $Q_1$ (kPa) & $Q_2$ (kPa) & $Q_3$ (kPa) \\
\hline
Lipid rich area (LpRi) & 5.5 ± 3.5 & 0.6–13.7 (18 sites) & 3.2 & 5.0 & 6.8 \\
Cellular fibrosis (CeFb) & 10.4 ± 5.7 & 1.6–25.0 (74 sites) & 6.3 & 9.9 & 13.8 \\
Hypocellular fibrosis (HyFb) & 59.4 ± 47.4 & 5.6–233.8 (97 sites) & 21.8 & 50.0 & 80.1 \\
\hline
\end{tabular}
\caption{Table 1}
\end{table}
et al. (2006) reported that elasticity modulus of fibrotic scar in infarcted rat myocardium is in the range 35–70 kPa. More recently, Barrett et al. (2009) performed indentation tests on fibrous cap samples collected from human carotid plaque and obtained Young’s modulus values in the range 21–300 kPa. In fact, a survey of the literature reveals that the wide range of Young’s modulus reported for atherosclerotic tissues remains to be clarified, notably by considering the differing methods used, the types of artery and the anisotropic properties of the tissue.

For mixture of lipids with varying cholesterol concentrations, Loree et al. (1994b) reported storage modulus at 1 Hz in the range of 0.3 kPa. Assuming that the lipid pool is isotropic and incompressible, derivation of Young’s modulus from the dynamic shear modulus gives elasticity values in the range of 0.9 kPa, which compares favorably with the elastic modulus we derived for lipid rich areas \( (E_{LpRi} = 5.5 \pm 3.5 \text{ kPa}) \) probed in situ.

In conclusion, this study took benefit of AFM methodology to obtain a spatial resolution of atherosclerotic plaque stiffness that successfully depicts the heterogeneous arrangement of elastic moduli within different regions of interest in a pre-clinical murine model of atherosclerosis. This approach of combined staining and biomechanical characterization has the potential to reinforce our knowledge on the correlation between mechanical stress and cellular processes. Interestingly, Young’s moduli of mouse plaque components were found to be lower than those observed in human plaques (Finet et al., 2004). The precise quantification of plaque stiffness heterogeneity presented here would allow to prospectively a better understanding of mechanobiological responses leading to plaque remodeling and rupture in mouse and human, according to both internal and external mechanical constraints imposed by vascular shear stress and blood pressure.

Acknowledgments

This work is supported by a grant from the French Agence Nationale de la Recherche (ANR, ATHEBIOMECH project 06-BLAN-0263). We are grateful to Dr. Agnès Piednoir (Lab. PMCN, Physique de la matière condensée et nanostructures, CNRS UMR 5586, Univ. C. Bernard, Lyon) for providing gold standard calibrated AFM probes.
References


