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Local axial compressive mechanical properties of human carotid atherosclerotic plaques—characterisation by indentation test and inverse finite element analysis

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ABSTRACT

The fibrous cap of an atherosclerotic plaque may be prone to rupture if the occurring stresses exceed the strength of the cap. Rupture can cause acute thrombosis and subsequent ischaemic stroke or myocardial infarction. A reliable prediction of the rupture probability is essential for the appropriate treatment of atherosclerosis. Biomechanical models, which compute stresses and strain, are promising to provide a more reliable rupture risk prediction. However, these models require knowledge of the local biomechanical properties of atherosclerotic plaque tissue. For this purpose, we examined human carotid plaques using indentation experiments. The test set-up was mounted on an inverted confocal microscope to visualise the collagen fibre structure during the tests. By using an inverse finite element (FE) approach, and assuming isotropic neo-Hookean behaviour, the corresponding Young's moduli were found in the range from 6 to 891 kPa (median 30 kPa). The results correspond to the values obtained by other research groups who analysed the compressive Young's modulus of atherosclerotic plaques. Collagen rich locations showed to be stiffer than collagen poor locations. No significant differences were found between the Young's moduli of structured and unstructured collagen architectures as specified from confocal collagen data. Insignificant differences between the middle of the fibrous cap, the shoulder regions, and remaining plaque tissue locations indicate that axial, compressive mechanical properties of atherosclerotic plaques are independent of location within the plaque.

1. Introduction

Atherosclerotic plaque rupture is the main cause of ischaemic stroke and myocardial infarction. Plaque rupture can lead to thrombus formation on the disrupted plaque surface and subsequent embolisation of thrombus into the distal vessels or to acute vessel occlusion. Rupture prone plaques are characterised by the presence of inflammatory cells, intraplaque haemorrhage and a lipid rich necrotic core (LRNC) covered by a thin fibrous cap. A reliable prediction model of cap rupture has a big impact on the treatment of atherosclerosis and atherosclerosis-related diseases (Rothwell and Warlow, 1999). Currently, the used methods to estimate plaque rupture is merely based on geometrical parameters, whereas biomechanical models have shown to provide a better risk assessment (Akyildiz et al., 2011; Finet et al., 2004; Hayenga et al., 2011; Salunke and Topoleski, 1997; Tracqui et al., 2011). However, the results of these models strongly depend on material properties of individual plaque components. Therefore, these models will benefit from specific knowledge of material properties of individual plaque components.

Experimental data on the mechanical properties of atherosclerotic tissue are scarce and show large variability, ranging from very soft (30–40 kPa, Lee et al. 1992; Barrett et al., 2009) to very stiff (of order 1 MPa, Lee et al., 1991; Loree et al., 1994; Ebenstein et al., 2002; Holzapfel et al., 2004). Moreover, most of the data represent only the average global stiffness of the plaque tissue tested and do not distinguish between different components within a plaque.

Recently, an experimental technique was developed by Cox et al. (2006, 2008), combining micro-indentation tests on soft

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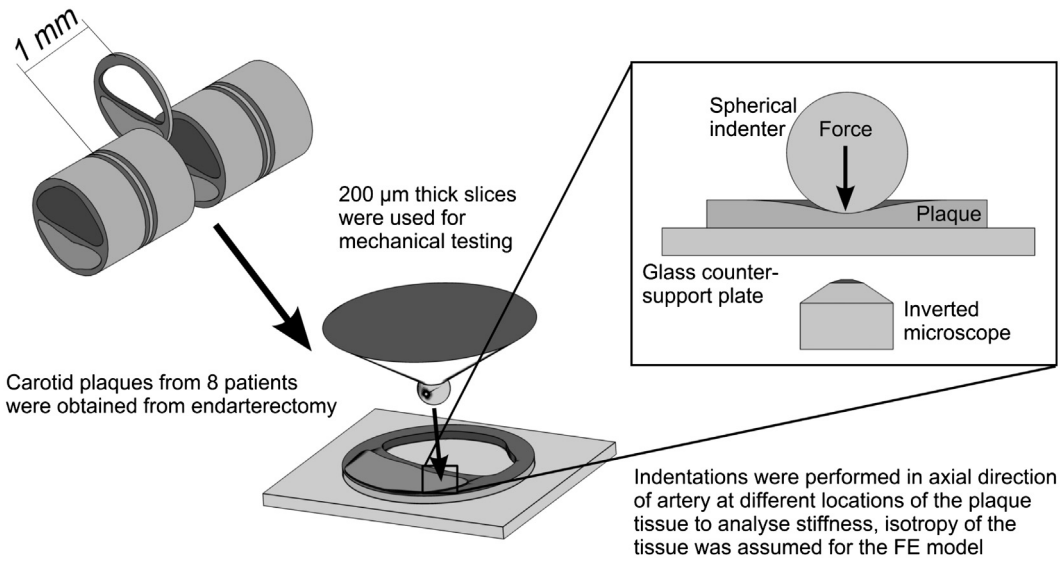

Fig. 1. Sectioning of plaque tissues with a cryotome to create 200 μm thick slices for mechanical testing. Depending on the axial length of the plaque, 6–13 slices were obtained. Adjacent slices are used for histology.

biological materials with confocal laser scanning microscope imaging. We apply this technique to investigate the compressive Young's moduli of different plaque components in the axial direction.

2. Methods

2.1. Preparing plaque tissue

Eight carotid artery endarterectomy specimens were obtained from eight symptomatic patients (2 female, 6 male, age 59 to 87). All plaques had ≥70% stenosis and low calcium content on preoperative CT angiography. Approval was given by the Institutional Review Board of the Erasmus MC, and informed consent was obtained. The plaques were snap-frozen, using liquid nitrogen and stored at −80°C. In a later stage, the plaques were sectioned, using a Leica cryotome at −20°C. Slices of 200 μm thicknesses were obtained with 1 mm spacing in between for the indentation experiments (Fig. 1).

Distal and proximal to each test section, slices of 5 μm thickness were cut for histology. A Gomori trichrome staining was applied on these slices. This stains collagen green/blue, muscle cells red, and nuclei black/blue. The histological images were used to determine indentation locations by visual registration before the experiments.

Prior to the mechanical testing, the 200 μm thick sections were thawed to room temperature and stained overnight using a fluorescent CNA35-OG488 probe (Nash-Krahn et al., 2006). This fluorescent staining was applied to visualise the collagen architecture of the plaque tissue.

2.2. Indentation test and imaging

To analyse the local mechanical properties of a plaque tissue, an existing indentation test set-up (Cox et al., 2008) was adapted, using a surface force apparatus developed by Vaenkatesan et al. (2006). At each testing location at least three consecutive indentations were performed using a spherical indenter with a diameter of 2 mm. During indentation the force response and the indentation depth were recorded. As described in Cox et al. (2010), the first indentation was considered as preconditioning and these results were not included. The measurements at the same indentation location were averaged. An inverted confocal laser scanning microscope (magnification 10x, excitation 488 nm, emission 500 nm high-pass), located underneath the set-up, was used to visualise the collagen structure, which were fluorescently stained (Fig. 1 and 2, Nash-Krahn et al., 2006; Boerboom et al., 2007). More detailed information regarding the indentation set-up can be found in Cox et al. (2005).

2.3. Data analysis

A 3D finite element model was created to simulate the indentation experiments, as previously described by Cox et al. (2008). The behaviour of the tissue was described with an isotropic incompressible Neo-Hookean model. The local shear modulus G at the test location was estimated by fitting the model to the experimental force-indentation-depth curve. The force response of up to 30% indentation of the tissue thickness was used for the parameter identification (Fig. 3). Simulations confirmed that the circumferential strain at 30% indentation of tissue thickness was about 20%, which corresponds to the upper limit of physiological strain range. The contact radius between indenter and tissue was about 0.4 mm². To fit the experimental data with the simulated data, the least-square method was used. To compare the results obtained in this study with the results in literature the Young's modulus E was calculated from the shear modulus G with

$$E = 3G \tag{1}$$

Measurement positions were classified by their indentation location and collagen structure. Based on the collagen structure, three different types of collagen architectures were distinguished using the confocal microscope (Fig. 2). Following the approach of Timmins et al. (2010) and Ng and Swartz (2006), a customised MATLAB script was generated to estimate the alignment index (AI) of the collagen fibres. The AI was given by

$$AI = \frac{\delta/(\Delta+\delta)}{\delta_{IR}/(\Delta+\delta)_{IR}} = \frac{\delta/(\Delta+\delta)}{40/180}, \tag{2}$$

where δ described the sum of frequencies of fibres within $\pm 20^\circ$ of the preferred fibre alignment (PFA) and Δ was the sum of frequencies of the remaining fibres outside of this range. The sums of frequencies δ_{IR} and Δ_{IR} described the corresponding sum of frequencies for an ideal random (IR) distribution, where the fibre dispersion is isotropic. These sum of frequencies were per definition $\delta_{IR}=40$ and $\Delta_{IR}=140$.

To decide if the collagen distribution was structured or unstructured an arbitrary threshold of $AI=1.4$ was chosen. Therefore, locations which showed a high amount of collagen fibres with a clear alignment ($AI > 1.4$) of the fibres were classified as dense structured collagen areas (SC). Positions with high amounts of collagen, but no clear alignment of the fibres ($AI < 1.4$), were characterised as loose unstructured collagen locations (UC). Collagen poor areas, primarily in the lipid rich necrotic core (LRNC) region, were classified as CP.

Each slice was indented at one to eight different locations. The locations were classified as middle of the fibrous cap, shoulder region of the fibrous cap, lipid rich necrotic core (LRNC) region and intima (remaining arterial wall regions, Fig. 4).

After the measurements the homogeneity at the tissue testing locations were examined based on the confocal images. Two observers (CKC and ACA), who were blinded to the stiffness results, conducted this evaluation. The following criteria led to the exclusion of the results from further analysis:

- Presence of debris or other foreign material, which were not identified as collagen fibres.
- Ruptured collagen fibres.
- Gap in the tissue which did not correspond to the structure of the surrounding tissue.
- Folded tissue, which can lead to slipping of the tissue influencing the stiffness results.

Statistical analysis was conducted to compare stiffness between indentation locations and between collagen types. The stiffness results showed a non-Gaussian