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Research Paper

Local anisotropic mechanical properties of human carotid atherosclerotic plaques – Characterisation by micro-indentation and inverse finite element analysis

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ABSTRACT

Biomechanical models have the potential to predict failure of atherosclerotic plaques and to improve the risk assessment of plaque rupture. The applicability of these models depends strongly on the used material models. Current biomechanical models employ isotropic material models, although it is generally accepted that plaque tissue behaves highly anisotropic. The aim of the present study is to determine the local anisotropic mechanical properties of human atherosclerotic plaque tissue by means of micro-indentation tests. The indentation was performed on top of an inverted confocal microscope allowing the visualisation and quantification of the collagen fibre deformations perpendicular to the indentation direction of the plaque. Based on this, the anisotropic properties of plaque tissue perpendicular to the indentation direction (middle of the fibrous cap, shoulder of the cap, remaining intima tissue) were derived. There were no significant differences between the different indentation locations for the fibre stiffness (total median 80.6 kPa, 25th–75th percentile 17.7–157.0 kPa), and fibre dispersion.

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1. Introduction

Atherosclerosis is a disorder of the arterial wall, characterised by wall thickening due to accumulation of cholesterol, macrophages, and smooth muscle cells. Eventually, this process can result in a rupture-prone atherosclerotic plaque. When an atherosclerotic plaque has a high risk of rupture, it is called a vulnerable plaque. A vulnerable plaque contains a large lipid rich necrotic core, which is covered by a thin fibrous cap, macrophages and frequently haemorrhage. The thin fibrous cap separates the thrombogenic lipid core from the blood stream and is prone to rupture. Plaque rupture can lead to thrombus formation and acute vessel occlusion or embolisation of plaque debris and/or thrombus in the distal vessel bed. If this happens in the carotid artery, it may lead to an transient ischaemic attack or an ischaemic stroke. In fact, carotid plaque rupture is a major cause of ischaemic strokes (Yuan et al., 2002).

Reliable diagnostic methods for plaque rupture are desired. Current methods are based on morphological plaque parameters, such as the degree of stenosis. However, studies showed that better risk assessment methods for plaque rupture are required (Tavora et al., 2010). From a mechanical point of view plaque rupture occurs when mechanical stresses exceed the strength of the plaque (Akyildiz et al., 2011; Finet et al., 2004; Hayenga et al., 2011; Salunke and Topoleski, 1997; Tracqui et al., 2011). Biomechanical models are able to compute stresses and strains of an atherosclerotic plaque, which allows prediction of local stress peaks and failure locations of the plaque (Salunke and Topoleski, 1997). However, the reliability of these computer models depends strongly on the applied material models. Currently, the material models mostly comprise isotropic material behaviour of the plaque tissue, although it is generally accepted that atherosclerotic plaque tissue behaves highly anisotropic (Holzapfel et al., 2004). This is especially the case for plaque components with high collagen content, such as the fibrous cap. Therefore, including anisotropic model parameters will improve biomechanical models and may improve the risk assessment of atherosclerotic plaque rupture.

The aim of the present study was to measure local anisotropic mechanical behaviour of collagenous rich plaque components at large strains. An in-house developed, unique indentation test setup was used to perform mechanical testing. During the indentation, deformation of collagen fibres of plaque tissue perpendicular to the indentation direction was recorded via confocal microscopy. Using digital image correlation, displacements of collagen fibres were quantified. By means of inverse finite element analysis, anisotropic behaviour of collagenous atherosclerotic plaque tissue was characterised.

2. Methods

2.1. Sample preparation

The sample preparation and mechanical tests have been extensively described in Chai et al. (2013, 2014). Atherosclerotic plaques from five symptomatic patients (two females, three males, age 65–87 years) were obtained by carotid endarterectomy, after written consent was given. Before the mechanical tests, the samples were snap-frozen and stored at −80 °C. At a later stage, the obtained plaques were sliced using a cryotome to create 200 μm thick transversal cross-sections. These cross-sections were thawed and a fluorescent staining was applied to visualise collagen fibre types I and III (Nash-Krahn et al., 2006) in the atherosclerotic plaques so that the deformation of the fibres can be quantified during mechanical testing.

2.2. Indentation test, imaging, and quantification of plaque deformation

Plaque cross-sections were placed underneath the indentation test device (Chai et al., 2013). A sphere head with a diameter of 2 mm indented the tissue along the axial direction of the vessel at a quasi-static rate of 0.01 mm/s. The small sphere allowed a local measurement. The tests were conducted at room temperature and a phosphate buffer saline solution was used to prevent dehydration of the plaque tissue. The first indentation to half of the tissue thickness was applied as preconditioning. Afterwards, another indentation to half of the tissue thickness was applied while recording the force-response. The resolution of the force measurement was 15 μN, whereas the indentation measurement was performed at a resolution of 15 nm. For a detailed description of the indentation test, see Cox et al. (2005) and Vaenkatesan et al. (2006). The indentation test device was placed on top of an inverted confocal microscope, which allowed visualisation of collagen fibres in the plaque. The main collagen fibre direction and the collagen fibre dispersion were determined by Fourier component analysis (ImageJ 1.47n plug-in 'Directionality'). Confocal imaging also enabled visualisation of collagen fibre deformation perpendicular to the indentation direction. The tissue deformations in the bottom plane of the sample during indentation were recorded. Using digital image correlation (DIC) (Cox et al., 2008), it was possible to quantify collagen deformation during indentation (Fig. 1). For DIC the commercially available imaging software package ARAMIS (GOM mbh, Germany) was used. The subset size was 200 μm × 200 μm. The experimental setup allowed measuring the indentation force-response, collagen fibre displacement perpendicular to fibre alignment, and collagen fibre stretch (Fig. 1). These parameters were used as an input for the inverse finite element (FE) analysis to determine anisotropic mechanical properties.

2.3. Inverse finite element (FE) analysis

A table outlining all symbols and its definitions is included (Table 1). For the inverse FE analysis, the material model based on Driessen et al. (2008) was applied. Advanced atherosclerotic plaques consist of different plaque components. Therefore, it is important to address this issue by applying local measurements. As depicted in Fig. 2, indentations were performed in the middle of the fibrous cap (shown in green, $n=7$), at the shoulder region of the cap (shown in red, $n=10$), and at the remaining diseased intima regions (shown in blue, $n=11$).

Indentations were conducted at different plaque locations. The testing locations were pre-selected based on histology