Journal of Biomechanics 47 (2014) 784–792

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Compressive mechanical properties of atherosclerotic plaques—Indentation test to characterise the local anisotropic behaviour

Chen-Ket Chai [a,*,1], Lambert Speelman [b,1], Cees W.J. Oomens [a], Frank P.T. Baaijens [a]

[a] Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands
[b] Department of Biomedical Engineering, Thoraxcentre, Erasmus Medical Centre, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Accepted 13 January 2014

Keywords:
Atherosclerosis
Compressive properties
Plaque rupture
Indentation
Finite element method

ABSTRACT

Accurate material models and associated parameters of atherosclerotic plaques are crucial for reliable biomechanical plaque prediction models. These biomechanical models have the potential to increase our understanding of plaque progression and failure, possibly improving risk assessment of plaque rupture, which is the main cause of ischaemic strokes and myocardial infarction. However, experimental biomechanical data on atherosclerotic plaque tissue is scarce and shows a high variability. In addition, most of the biomechanical models assume isotropic behaviour of plaque tissue, which is a general over-simplification. This review discusses the past and the current literature that focus on mechanical properties of plaque derived from compression experiments, using unconfined compression, micro-indentation or nano-indentation. Results will be discussed and the techniques will be mutually compared. Thereafter, an in-house developed indentation method combined with an inverse finite element method is introduced, allowing analysis of the local anisotropic mechanical properties of atherosclerotic plaques. The advantages and limitations of this method will be evaluated and compared to other methods reported in literature.

1. Introduction

Atherosclerosis is a disorder of the arterial wall. The vessel wall is invaded by lipids and inflammatory cells that can eventually lead to formation of an atherosclerotic plaque. Some of these plaques develop into plaques that are vulnerable for plaque rupture. Such a vulnerable plaque consists of inflammatory cells, a lipid rich necrotic core, intra-plaque haemorrhage, and a thin fibrous cap separating the thrombogenic lipid core from the bloodstream (Schaar et al., 2004; Stary et al., 1992). In case of rupture of the thin fibrous cap, the lipid core comes into contact with the blood, causing luminal thrombus formation. This thrombus may cause a blockage in the vessels distal to the plaque. This is the major cause of ischaemic stroke and myocardial infarction (Sakakura et al., 2013).

Current methods to assess plaque rupture risk are mostly based on general risk factors (age, hypertension, and familial arterial diseases), and on geometrical plaque features (stenosis degree, intima-media thickness, and irregular, ulcerated plaque morphology) (Naghavi, 2010). It has been shown that these risk factors are insufficient for predicting future plaque rupture events. A reliable computational model to predict cap rupture may, therefore, add to the diagnosis and treatment of atherosclerotic plaques. Since fibrous cap rupture occurs when the stresses in the cap exceed the strength of the cap, biomechanical plaque modelling has the potential to improve risk assessment of plaque rupture (Sadat et al., 2011; Salunke and Topoleski, 1997). Biomechanical plaque studies have revealed that patient-specific plaque models can accurately predict local stress peaks and rupture locations (Akyildiz et al., 2011, this issue; Gillard, 2007; Loree et al., 1992, 1994; Nieuwstadt et al., in press; Speelman et al., 2011; Tang et al., 2005, 2009). However, the stress results from these biomechanical models strongly depend on the material models and the parameters used (Akyildiz et al., 2011; Williamson et al., 2003). Determining the mechanical behaviour of the different plaque components is, therefore, a necessity.

Mechanical characterisation of plaque tissue is frequently done using (uni-)axial tensile tests (Lawlor et al., 2011; Maher et al., 2009), which is discussed by Walsh et al. (this issue) in this special issue. Although plaque tissue *in vivo* experiences circumferential stretching during blood pressure pulsation, the tissue is also radially compressed during this pulsation. Therefore, also compression tests are

* Correspondence to: Eindhoven University of Technology, Department of Biomedical Engineering, PO Box 513, GEM-Z 4.11, 5600 MB Eindhoven, The Netherlands. Tel.: +31 40 247 3047; fax: +31 40 244 7355.
E-mail address: C.Chai@tue.nl (C.-K. Chai).
[1] These authors contributed equally to this work.

0021-9290/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbiomech.2014.01.018

physiologically relevant to determine the mechanical behaviour of plaque tissue.

The aim of this review paper is to give an overview of the methods used in literature to measure mechanical properties of atherosclerotic plaque tissue using compression experiments. In literature, studies were identified in which the compressive properties of human, porcine or murine plaques were tested, harvested from the aorta, iliac, femoral, or carotid arteries. Plaque tissue was tested using unconfined compression, micro-indentation, or nano-indentation. The results from the different studies and testing techniques will be compared. The advantages and disadvantages of each technique will be discussed. In case the stiffness data was presented non-conventionally, representative figures from the papers were digitised and used to fit a neo-Hookean material model and to extract tangential stiffness values.

After a summary of the literature on compressive plaque properties, an in-house developed micro-indentation test will be presented for the characterisation of anisotropic properties of plaque tissue. The relevance and limitations of this method will be discussed.

2. Compressive properties of plaque tissue

2.1. Unconfined compression

Unconfined compression tests are one of the most popular methods to determine mechanical behaviour of materials. The samples are mounted between two metal plates, of which the top plate is stationary and attached to a load measuring device. Generally, during unconfined compression, the tested tissue is smaller in size than the compression plates. The bottom plate can be raised and lowered with a pre-set speed to pre-set positions. Both displacement and force can be used as loading conditions. With unconfined compression tests, compressive mechanical plaque properties can be determined at large, physiologically relevant strain. It is a relatively straightforward test that allows both static and dynamic loading conditions. Using static loading conditions, material stiffness can be determined, while frequency-dependent dynamic characteristics can be identified using cyclic unconfined compression loading conditions at different frequencies.

2.1.1. Human aortic plaques

Lee et al. (1991) evaluated radial compressive plaque properties, by compressing 27 fibrous caps from 14 abdominal aorta plaques using a 7 mm diameter cylindrical steel plate. Caps were classified as cellular ($n=7$), hypo-cellular ($n=9$), or calcified ($n=11$) based on histological examination. The tests were conducted at room temperature within 16 h of patient death. The dynamic stiffness of the test samples (thickness 1.1 ± 0.2 mm) was evaluated by applying a static compressive stress of 9.3 kPa in the radial direction first and, after a resting period to reach static equilibrium, a dynamic stress with an amplitude of 0.5 kPa at different frequencies afterwards. Stiffness values increased with increasing frequency; however, the change was less than 10% between 0.5 and 2 Hz. As the stiffness was determined at a fixed load, the samples were subjected to different compression levels ($17 \pm 6\%$ for cellular, $7 \pm 1\%$ for hypo-cellular, and $1.2 \pm 0.2\%$ for calcified samples). The dynamic stiffness was 510 ± 220 kPa for cellular, 900 ± 220 kPa for hypo-cellular and 2.2 ± 1.0 MPa for calcified samples.

The same group studied the relation between compressive mechanical properties and intravascular ultrasound classification of aortic plaques, with a static measurement protocol, using the same set-up (Lee et al., 1992). An initial compressive stress of 4.0 kPa was applied until a static equilibrium was reached. Thereafter, the compressive stress was increased up to 12 kPa. Strain and creep-times were recorded for this step and a stiffness modulus was determined. The compression strain was $24 \pm 11\%$ for non-fibrous caps, $11 \pm 5\%$ for fibrous caps and $3 \pm 2\%$ for calcified caps. Creep times varied from 20 min for calcified samples, 50 min for fibrous samples, and 80 min for non-fibrous samples. Non-fibrous samples had a stiffness modulus of 41 ± 18 kPa. The stiffness modulus for fibrous and calcified caps was 82 ± 33 kPa and 355 ± 245 kPa, respectively.

Although the static loading stress was not equal in both studies (9.3 kPa versus 8 kPa), the strain levels were comparable for the plaque samples. However, the reported static stiffness values were about one order lower compared to the reported dynamic stiffness values. Lee et al. (1992) suggested that these differences may be attributed to the non-elastic mechanical behaviour of the tissue, as a different loading stress was applied in both studies (9.3 ± 0.5 kPa versus 8 kPa). Additionally, visco-elastic behaviour of the cap may play a role in the dynamic stiffness results. This is supported by the relatively long creep-times, which were reported by Lee et al. (1992).

Walraevens et al. (2008) used unconfined compression to test the compressive mechanical properties of atherosclerotic calcified human aortas ($n=19$), obtained from aneurysm repair surgeries. The aortas were cut into 10 mm × 10 mm strips, with an average thickness of 1.84 ± 0.28 mm. The aortas were tested at room temperature using an impermeable pounder with a diameter of 6 mm. The *E*-moduli obtained at 10% strain were 321 ± 258 kPa for calcified human plaques, which was similar to the calcified samples from Lee et al. (1992), although the amount of compression was different (10% versus $3 \pm 2\%$).

2.1.2. Human iliac and femoral plaques

Topoleski et al. (1997) investigated radial compressive behaviour of aortoiliac plaques by quasi-static compressing samples at 37 °C using parallel flat acrylic fixtures (6.35 mm in radius) (Topoleski et al., 1997; Topoleski and Salunke, 2000). Non-ulcerated lesions ($n=24$) were obtained from 6 autopsies and stripped from the remaining vessel wall. The samples (5 mm × 5 mm, 2.4 ± 0.7 mm thickness) underwent two 15-cycle loading phases up to 350 kPa with a 10–15 min unloaded rest period in between. Three types of plaques were identified based on histological features, which all showed a distinct mechanical behaviour in terms of repeatability and recoverability. The maximum compressive strain at 350 kPa loading stress was $70 \pm 11\%$ for atheromatous samples, $54 \pm 9\%$ for fibrous samples, and $14 \pm 9\%$ for calcified samples. Tangential stiffness values were extracted from the representative curves. Atheromatous samples showed the most compliant response (stiffness < 10 kPa for compression values up to 25%). Fibrous samples were stiffer; however, much softer than calcified samples (< 10 kPa versus 830 kPa at 5% compression and 85 kPa versus 13 MPa at 20% compression).

Using the same approach, Salunke et al. (2001) investigated the compressive stress–relaxation behaviour of aortoiliac plaques in radial direction. Atherosclerotic plaques (5 calcified, 7 fibrous, and 6 atheromatous samples) were obtained post-mortem. After two 15-cycle preconditioning phases, the samples (5 mm × 5 mm, 1.5 ± 0.7 mm thickness) were subjected to three stress–relaxation phases, with 25% compression within 1 s between 16 mm diameter parallel plates. Stiffness values were determined from representative curves. Fibrous plaques and calcified plaques showed similar stiffness values (100 kPa versus 70 kPa at 5% compression and 900 versus 1000 kPa at 20% compression), while atheromatous tissue had a lower average stiffness (25 kPa at 5% compression and 100 kPa at 20% compression). Compared to Topoleski et al. (1997), the dynamic stiffness of fibrous and atheromatous plaque tissue was higher than the static stiffness,