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# Material properties of components in human carotid atherosclerotic plaques: A uniaxial extension study

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ABSTRACT

Computational modelling to calculate the mechanical loading within atherosclerotic plaques has been shown to be complementary to defining anatomical plaque features in determining plaque vulnerability. However, its application has been partially impeded by the lack of comprehensive knowledge about the mechanical properties of various tissues within the plaque. Twenty-one human carotid plaques were collected from endarterectomy. The plaque was cut into rings, and different type of atherosclerotic tissues, including media, fibrous cap (FC), lipid and intraplaque haemorrhage/thrombus (IPH/T) was dissected for uniaxial extension testing. In total, 65 media strips from 17 samples, 59 FC strips from 14 samples, 38 lipid strips from 11 samples, and 21 IPH/T strips from 11 samples were tested successfully. A modified Mooney–Rivlin strain energy density function was used to characterize the stretch–stress relationship. The stiffnesses of media and FC are comparable, as are lipid and IPH/T. However, both media and FC are stiffer than either lipid or IPH/T. The median values of incremental Young's modulus of media, FC, lipid and IPH/T at $\lambda = 1$ are 290.1, 244.5, 104.4, 52.9, respectively; they increase to 1019.5, 817.4, 220.7 and 176.9 at $\lambda = 1.1$; and 4302.7, 3335.0, 533.4 and 268.8 at $\lambda = 1.15$ (unit, kPa; $\lambda$, stretch ratio). The material constants of each tissue type are suggested to be: media, $c_1 = 0.138$ kPa, $D_1 = 3.833$ kPa and $D_2 = 18.803$; FC, $c_1 = 0.186$ kPa, $D_1 = 5.769$ kPa and $D_2 = 18.219$; lipid, $c_1 = 0.046$ kPa, $D_1 = 4.885$ kPa and $D_2 = 5.426$; and IPH/T, $c_1 = 0.212$ kPa, $D_1 = 4.260$ kPa and $D_2 = 5.312$. It is concluded that all soft atherosclerotic tissues are non-linear, and both media and FC are stiffer than either lipid or IPH/T.


## 1. Introduction

Stroke is the third leading cause of death globally [1], with carotid atherosclerotic disease responsible for 25–30% of cerebrovascular ischemic events in western nations [2]. Currently, carotid luminal stenosis is the only validated diagnostic criterion for risk stratification, but this criterion becomes less reliable in patients with mild to moderate stenoses. Clinical trials have shown that carotid endarterectomy (CEA) provides maximum benefit for patients with significant carotid stenoses ($\geqslant$70%), but the overall benefit of CEA becomes negligible when stenosis severity is reduced (<70%). Importantly, the majority of clinical events occur in patients with mild to moderate carotid stenoses [3,4]. Novel non-invasive diagnostic screening methods are urgently needed to identify vulnerable plaques earlier in an attempt to avoid acute ischemic events.

Atherosclerotic plaques are multicomponent structures composed of some, or all, of the following components: lipid, calcium, plaque haemorrhage (PH) and fibrous cap (FC). A typical vulnerable carotid plaque underlying a clinical event is frequently characterized by the presence of PH and FC rupture. These features can be quantified by in vivo medical imaging modalities, including high-resolution, multi-contrast magnetic resonance imaging (MRI) [5–7], and used to predict events in symptomatic [8,9] and asymptomatic [10,11] patients. In a meta-analysis of 31 histological studies, the prevalence of PH was increased in symptomatic vs. asymptomatic patients [12]. Similarly, in a recent meta-analysis of eight clinical studies of 689 patients, the hazard ratio of MRI-depicted PH in symptomatic patients was 11.7 [13]. FC rupture is

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also a common feature in symptomatic patients with a prevalence of ~60% [14]. In vivo imaging-based studies have demonstrated an association between FC rupture and subsequent events in symptomatic patients [8]. Event rates are increased when combinations of these two features are present [15]. Although 60–70% symptomatic patients exhibit PH or FC rupture at baseline [12,14,16], only ~15% will experience a recurrent event at one year [17,18]. From these results, it is clear that image-detected PH or FC rupture alone, or in combination, cannot serve as a robust marker for prospective cerebrovascular risk, and additional analyses or biomarkers are required.

Carotid atherosclerotic plaques continually undergo large deformations as a result of blood pressure and flow. From a material viewpoint, rupture could possibly occur when the external loading exceeds the plaques' material strength. Therefore, biomechanical analysis may provide complementary information to plaque structure and luminal stenosis in determining vulnerability. Previous results have shown that most rupture sites are also sites of increased mechanical stress [19–22]. Calculating mechanical stress within FC may help differentiate symptomatic and asymptomatic individuals [23–25] and could provide incremental information to predict subsequent ischemic cerebrovascular events in symptomatic patients in the carotid [26] and coronary [27]. These findings suggest that plaque morphology features and critical mechanical condition should be considered in an integrative way for a more accurate vulnerability assessment.

However, the clinical applicability of numerical simulations has been partially impeded by a lack of data regarding the mechanical properties of various atherosclerotic plaque components. Direct material measurements from human atherosclerotic tissues are limited (readers are referred to summaries on the direct measurements with atherosclerotic tissue in Refs. [28,29]). In particular, the material properties of lipid and intraplaque haemorrhage/thrombus (IPH/T) have never been explored. This study presents data on the material behaviour of media, FC, lipid and IPH/T using uniaxial extension testing.

## 2. Materials and methods

### 2.1. Tissue preparation and testing

Twenty-one patients with symptomatic carotid atherosclerotic diseases who were scheduled for CEA were recruited consecutively. The patient demographics are listed in Table 1. The study was approved by the local ethics committee, with all patients giving written informed consent. Carotid plaques were collected during surgery and banked in liquid nitrogen for <4 months prior to testing. Cryoprotectant solution (20% dimethylsulfoxide (DMSO) in 5% human albumin solution) added to a final concentration of 10% DMSO was used to prevent ice crystals damaging the tissue. Prior to testing, samples were thawed in a 37 °C tissue bath and cut into rings 1–2 mm thick perpendicular to the blood flow direction from proximal (closer to the heart) to distal, using a scalpel. Approximately 10 rings were obtained from each plaque, and alternate rings were used for material testing. Each ring was further dissected to separate different atherosclerotic tissue components along the ring under a stereo microscope using fine ophthalmic clamps and scissors (Figs. 1–3). The tissue strips were prepared carefully to minimise variation in width and thickness along the length. FC and media were relatively easy to identify and separate; lipid appeared yellow or yellowish, based on visual inspection (Fig. 2); and red or erythroid brown was classified as IPH or thrombus (Fig. 3).

**Table 1**
Patient demographics ($n$ = 21).

| | |
|---|---|
| Male, $n$ (%) | 18 (85.7) |
| Age (mean ± SD) | 68.2 ± 7.4 |
| Hypertension, $n$ (%) | 19 (90.5) |
| Coronary artery disease, $n$ (%) | 6 (28.6) |
| Diabetes mellitus, $n$ (%) | 4 (19.0) |
| Previous use of statin, $n$ (%) | 12 (57.1) |
| NASCET defined stenosis (%) | 72.3 ± 17.0 |

An in-house-designed tester, consisting of a stepper motor (Miniature Steel Linear Stages, Newport Corporation, USA), load cell (custom designed), camera (PixeLink PL-B776U 3.1 MP USB2 Colour Camera, PixeLINK, Canada) and controlling system developed in LabView 2011 (National Instruments, USA), was used to perform the uniaxial extension tests. The position resolution of the stepper motor was 0.1 μm; the precision of the load cell was 0.0005 N, and the measuring range was 2 N; the image size was 2048 × 1536 pixels, with an 80 × 60 $mm^2$ field of view. The tissue strip was mounted on the tester using modified 6-cm straight haemostatic clamps (Shanghai Medical Instruments (Group) Ltd., Corp. China). The clamped section of each end was 1–1.5 mm. After five preconditioning cycles (by moving one of the clamps 2.5% of the total distance between the two clamp ends at a speed of 0.05 mm $s^{-1}$), the testing was performed with a speed of 0.01 mm $s^{-1}$ in a 37 °C saline bath with a 0.005 N pre-loading. Waterproof black ink markers were placed on the surface to trace local displacement. The centre of each marker was identified, and the local stretch ratio was calculated from the distance between the centres. The Cauchy stress was converted from the measured force signal using the strip thickness, the width at rest and the stretch ratio, with the material being assumed to be incompressible.

### 2.2. Data processing

A modified Mooney–Rivlin strain energy density function was used to characterize the stretch–stress relationship of each tissue type:

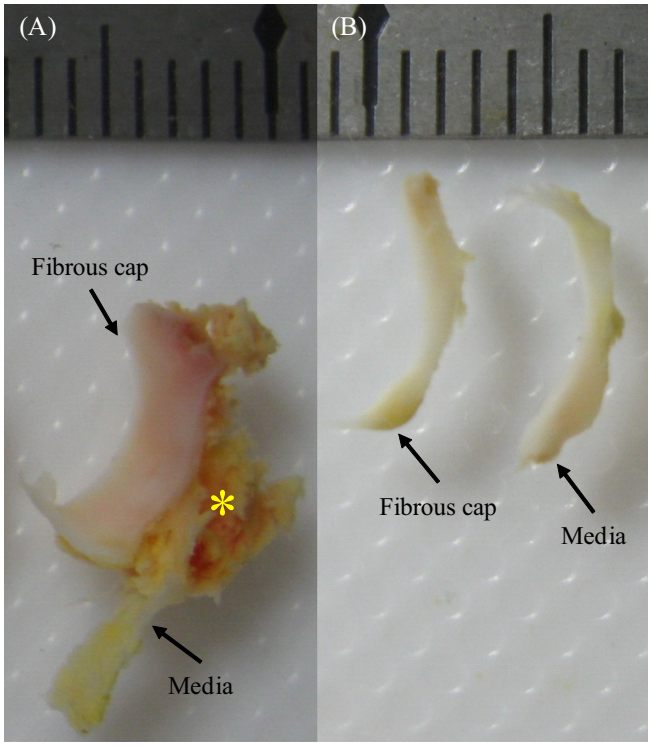


**Fig. 1.** A representative tissue section with FC and media: (A) the intact section with FC, media and lipid marked by a yellow asterisk; (B) isolated tissue strips of FC and media.