Journal of the Mechanical Behavior of Biomedical Materials

The Compositions of the Vulnerable Plaque and its Effect on Arterial Waveform

--Manuscript Draft--

Manuscript Number:	JMBBM-D-20-01258R2
Article Type:	VSI:Fracture of Biomaterials
Section/Category:	Other Biological/Natural Materials
Keywords:	Carotid plaque; Plaque compositions; Wave intensity analysis; Wave separation
Corresponding Author:	Jiling Feng, PhD, MSc, BEng, PgCAP Manchester Metrololitan University UNITED KINGDOM
First Author:	Jiling Feng, PhD, MSc, BEng, PgCAP
Order of Authors:	Jiling Feng, PhD, MSc, BEng, PgCAP
	Mohamed Abdulsalam, MSc
Abstract:	Carotid plaque composition is a key factor of plaque stability and it carries significant prognostic information. The carotid unstable plaques are characterized by a thin fibrous cap (FC) with large lipid core (LC), while stable plaques have a thicker FC and less LC. Identifying the percentage of plaque compositions could help surgeons to make a precise decision for their patients' treatment protocol. This study aims to distinguish between stable and unstable plaque by defining the relationship between plaque composition and arterial waveform non-invasively. An in-vitro arterial system, composed of a Harvard pulsatile flow pump and artificial circulation system, was used to investigate the effect of the plaque compositions on the pulsatile arterial waveforms. Five types of arterial plaques, composed of the LC, FC, Collagen (Col) and Calcium (Ca), were implemented into the artificial carotid artery to represent the diseased arterial system with 30% of blockage. The pulsatile pressure, velocity and arterial wall movement were measured simultaneously at the site proximal to the plaque. Non-invasive wave intensity analysis (Non-WIA) was used to separate the waves into forward and backward components. The correlation between the plaque compositions and the reflected waveforms was quantitatively analysed. The experimental results indicate that the reflected waveforms are strongly correlated with the plaque compositions, where the percentages of the Col are linearly correlated with the amplitude of the backward diameter (correlation coefficient, r = 0.74) and the lipid content has a strong negative correlation with the backward diameter (r = 0.82). A slight weak correlation exists between the reflected waveform and the percentage of Ca. The strong correlation between the compositions of the plaques with the backward waveforms observed in this study demonstrates that the components of the arterial plaques could be distinguished by the arterial waveforms. This finding might lead to a potential novel non-invasive clinical tool to determine the composition of the plaques and distinguish between stable and vulnerable arterial plaques at the early stage
Suggested Reviewers:	Liguo Zhao, PhD Professor, Loughborough University Faculty of Engineering: Loughborough University l.zhao@lboro.ac.uk Prof Liguo Zhao is a Professor in Wolfson School of Mechanical, Electrical and Manufacturing Engineering, Loughborough University with the expertise in the finite element modelling and experimental study for the soft tissue, particular in the damage mechanism analysis for arteries.
	Ferdinand Serracino-Inglott, PhD Professor, Manchester Royal Infirmary ferdinand.serracino-inglott@mft.nhs.uk Prof Ferdinand Serracino-Inglott is the vascular surgeon consultant in Manchester Royal Infirmary. He has a specialty for the vascular surgery in peripheral artery.
	Ye Li, PhD Research Fellow, King's College London ye.1.li@kcl.ac.uk Dr Ye Li is a research fellow in Faculty of Life Science and Medicine. She has an

	intensive experience on the clinical researches on the cardiovascular mechanics.
Response to Reviewers:	Response to Reviewers' Comments Reviewer #1 (1)"Thank you to the authors for taking the suggestions into account. Some minor comments: -Clarification was added on Non-wia by imaging and non-WIA for plaque detection vs other applications." Response: Thanks for reviewer's further comments for clarification of Non-WIA in different applications. It is emphasized here that WIA needs the original data of measured pressure and velocity for analysis, where Non-WIA needs the data of measured diameter and velocity for analysis. The later one can provide the analysis approach based on the original data measured non-invasively. (2)"- In Methods: I would explicitly specify that the pressure sensor is only used for controlling the in-vitro setup and not for WIA analysis." Response: It is correct. In this study, the pressure is measured to monitor the healthy pressure range which targets 120mmHg-80mmHg. The pressure can be used for WIA analysis but not for Non-WIA analysis. The pressure can be measured in in-vivo but needs to be measured invasively through tipped catheter pressure sensor. (3)- From answer to reviewers: "The effect of the location of the different compositions on the arterial waveforms will be further investigated" - This should also be added to the discussion section of the paper. Response: Thanks for the reviewer's suggestion. ""The effect of the location of the different compositions on the arterial waveforms will be further investigated" has been added to discussion section (page 7, 2nd paragraph, red font). (4)- More details to be added on clinical implementation, beyond the accuracy of ultrasound/MRI - how will the waveform be analysed after WIA is applied. For example, "stable plaque generated higher amplitude than unstable plaques," it is a very positive outcome that the plaque compositions are expressed in the waveform. However, in a patient, how is it known if there is a "stable" plaque or an "unstable" plaque present - since the plaque cannot be removed or replaced as in the in-vitro study - no "differential diagnosis" can be applied. Is there a particular waveform feature specific to the vulnerable plaque, but which is not present in the non-vulnerable plaque? The amplitude by itself may depend on a lot of other factors than plaque composition (bifurcations, stiffness, compensatory effects etc)- can a certain detected amplitude be evidence of an unstable plaque? While this study is relevant as it improves knowledge of plaque compositions and their relationship to waveform, it would be interesting to tackle this clinical question briefly in the discussion section, as an opportunity for future study. Response: Thanks for reviewer's comments and questions for how to identify the vulnerable plaques in clinical setting. The key information from this study is that the correlation of the backward arterial waveforms (amplitude) and compositions of different types of plaques. As is shown in the manuscript, the arterial waveforms for the diseased artery was compared with the arterial waveforms for healthy one. The most important thing is that arterial waveform for vulnerable plaque, characterised with high percentage of lipid core, is similar to the arterial waveform for healthy one, indicating the weak reflection caused by the vulnerable plaques. We hope that next step this approach can be used for in-vivo study regarding the correlation of arterial waveform and compositions of the plaque. I have no doubt that the arterial waveform in vivo study will have more complicated information than those observed in vitro study. The possible application of the approach to clinical setting and potential challenges have been discussed (page 6, red font). (5)- I recommend another proof-read as some statements can be corrected grammatically or improved. Response: Thanks for reviewer's comments. The very carefully proof-reading has been done by all authors.

21/02/2021

Dear Editor and Reviewers,

We want to thank you for the email dated 10th Feb 2021 enclosing the reviewer' s comments for our manuscript *'The Compositions of the Vulnerable Plaque and its Effect on Arterial Waveforms'*. We have carefully reviewed the comments and have revised the manuscript accordingly.

Following this letter is our point-by-point response to the comments raised by the reviewers. The comments are reproduced, and our responses are given directly afterwards in italics. We have uploaded the revised manuscript with all the changes highlighted in the colour red.

We would like to thank you for allowing us to resubmit a revised copy of our manuscript and hope that the revised manuscript is accepted for publication in the Journal of the Mechanical Behavior of Biomedical Materials.

Sincerely yours,

Jiling Feng

_____
Dr. Jiling Feng
Department of Engineering
Manchester Metropolitan University
Chester Street
Manchester, UK, M1 5GD
Tel.: +441612471650
Email: j.feng@mmu.ac.uk

Response to Reviewers' Comments

Reviewer #1

(1) "Thank you to the authors for taking the suggestions into account. Some minor comments: -Clarification was added on Non-wia by imaging and non-WIA for plaque detection vs other applications."

Response: *Thanks for reviewer's further comments for clarification of Non-WIA in different applications. It is emphasized here that WIA needs the original data of measured pressure and velocity for analysis, where Non-WIA needs the data of measured diameter and velocity for analysis. The later one can provide the analysis approach based on the original data measured non-invasively.*

(2) "- In Methods: I would explicitly specify that the pressure sensor is only used for controlling the in-vitro setup and not for WIA analysis."

Response: *It is correct. In this study, the pressure is measured to monitor the healthy pressure range which targets 120mmHg-80mmHg. The pressure can be used for WIA analysis but not for Non-WIA analysis. The pressure can be measured in in-vivo but needs to be measured invasively through tipped catheter pressure sensor.*

(3) - From answer to reviewers: "The effect of the location of the different compositions on the arterial waveforms will be further investigated" - This should also be added to the discussion section of the paper.

Response: *Thanks for the reviewer's suggestion. ""The effect of the location of the different compositions on the arterial waveforms will be further investigated" has been added to discussion section (page 7, 2nd paragraph, red font).*

(4) - More details to be added on clinical implementation, beyond the accuracy of ultrasound/MRI - how will the waveform be analyzed after WIA is applied. For example, "stable plaque generated higher amplitude than unstable plaques," it is a very positive outcome that the plaque compositions are expressed in the waveform. However, in a patient, how is it known if there is a "stable" plaque or an "unstable" plaque present - since the plaque cannot be removed or replaced as in the in-vitro study - no "differential diagnosis" can be applied. Is there a particular waveform feature specific to the vulnerable plaque, but which is not present in the non-vulnerable plaque? The amplitude by itself may depend on a lot of other factors than plaque composition (bifurcations, stiffness, compensatory effects etc)- can a certain detected amplitude be evidence of an unstable plaque? While this study is relevant as it improves knowledge of plaque compositions and their relationship to waveform, it would be interesting to tackle this clinical question briefly in the discussion section, as an opportunity for future study.

Response: *Thanks for reviewer's comments and questions for how to identify the vulnerable plaques in clinical setting. The key information from this study is that the correlation of the backward arterial waveforms (amplitude) and compositions of different types of plaques. As is shown in the manuscript, the arterial waveforms for the diseased artery was compared with the arterial waveforms for healthy one. The most important thing is that arterial waveform for vulnerable plaque, characterised with high percentage of lipid core, is similar to the arterial waveform for healthy one, indicating the weak reflection caused by the vulnerable plaques. We hope that next step this approach can be used for in-vivo study regarding the correlation of arterial waveform and compositions of the plaque. I have no doubt that the arterial waveform in vivo study will have more complicated information than those observed in vitro study. The possible application of the approach to clinical setting and potential challenges have been discussed (page 6, red font).*

(5) - I recommend another proof-read as some statements can be corrected grammatically or improved.

Response: *Thanks for reviewer's comments. The very carefully proof-reading has been done by all authors.*

The Composition of Vulnerable Plaque and its Effect on Arterial Waveforms

M. Abdulsalam [a], J. Feng [a]

[a] Department of Engineering, Faculty of Science and Engineering, Manchester Metropolitan University, John Dalton – Chester St – M1 5GD, J.Feng@mmu.ac.uk

ARTICLEINFO

Keywords:
Carotid plaque;
Plaque compositions;
Wave intensity analysis;
Wave separation

ABSTRACT

Carotid plaque composition is a key factor of plaque stability and it carries significant prognostic information. The carotid unstable plaques are characterized by a thin fibrous cap (FC) $\leq 65\mu m$ with large lipid core (LC), while stable plaques have a thicker FC and less LC. Identifying the percentage of plaque compositions could help surgeons to make a precise decision for their patients' treatment protocol. This study aims to distinguish between stable and unstable plaque by defining the relationship between plaque composition and arterial waveform non-invasively. An *in-vitro* arterial system, composed of a Harvard pulsatile flow pump and artificial circulation system, was used to investigate the effect of the plaque compositions on the pulsatile arterial waveforms. Five types of arterial plaques, composed of the LC, FC, Collagen (Col) and Calcium (Ca), were implemented into the artificial carotid artery to represent the diseased arterial system with 30% of blockage. The pulsatile pressure, velocity and arterial wall movement were measured simultaneously at the site proximal to the plaque. Non-invasive wave intensity analysis (Non-WIA) was used to separate the waves into forward and backward components. The correlation between the plaque compositions and the reflected waveforms was quantitatively analysed. The experimental results indicate that the reflected waveforms are strongly correlated with the plaque compositions, where the percentages of the Col are linearly correlated with the amplitude of the backward diameter (correlation coefficient, r = 0.74) and the lipid content has a strong negative correlation with the backward diameter (r = 0.82). A slight weak correlation exists between the reflected waveform and the percentage of Ca. The strong correlation between the compositions of the plaques with the backward waveforms observed in this study demonstrates that the components of the arterial plaques could be distinguished by the arterial waveforms. This finding might lead to a potential novel non-invasive clinical tool to determine the composition of the plaques and distinguish between stable and vulnerable arterial plaques at the early stage

1. Introduction

Prediction of plaque progression is of essential significance to cardiovascular studies and disease diagnosis, prohibition and treatment. Atherosclerotic plaque is one of the arterial diseases which builds up in the arterial wall and can be identified by the composition of the plaque that includes lipid core (LC), calcium (Ca), collagen (Col) and other substances from the blood (Libby, et al. 2011). Atherosclerosis causes the narrowing of the carotid lumen leading to either occlusion of the blood flow to the brain cells or plaque rupture causing stroke event (Li et al. 2018). It causes around 4.1 million deaths per year, about 46% of all deaths in Europe (Nichols et al. 2013). Globally, almost 17.5 million died because of cardiovascular diseases, and this figure is expected to increase to around 24 million by 2030 (Stroke Association 2018). Stroke Association also expects that people over 45, who have first-time strokes, will increase by 59 % within the next 20 years. Several studies, such as by Li et al. (2018), have indicated that most ischemic strokes are correlated with carotid plaque.

Carotid plaques could be mainly classified as stable and vulnerable (Finn et al. 2010). Vulnerable plaques are often identified by a thin FC with a large LC and interstitial Col (Van Den Oord et al. 2014), which increases the rupture risk. However, stable plaques can be characterized by a dense FC thickness including smooth muscle cells in an extracellular matrix rich in type I and III Col that tends to be asymptomatic (Finn et al. 2010). The appearance of calcification in the atherosclerotic plaque is prevalent in the late-stage of carotid plaque and grows with age (Bentzon et al. 2014). The existence of Col and Ca in the plaque could increase the stiffness leading to stability in the plaque (Li et al. 2018). Although the recent clinical practices such as carotid endarterectomy rely heavily on the stenosis degree (Chan et al. 2014), most of stroke events occurred not due to lumen narrowing itself (Ammirati et al. 2015). Moreover, several histopathological studies have detected that stroke events can happen with plaque stenosis less than 30% and without any other detectible reason for their stroke (Wasserman et al. 2005).

Non-invasive imaging technique to evaluate the relationship between the behaviour of the plaque and the possibility of stroke event has been improved by applying Magnetic Resonance Image (MRI), Computed Tomography (CT), ultrasound and other sophisticated techniques (Underhill et al. 2010; Chan et al. 2014). However, to date, the effective non-invasive imaging technique to distinguish the vulnerable plaques in the carotid artery is still lacking.

Several kinds of research have attempted to study the diseased blood vessels non-invasively. For instance, distinguishing between diseased and healthy coronary arteries was investigated by Biglino et al. (2012 using arterial waveform. In addition, the effect of Ca and lipid of the carotid plaque on measured arterial waveform was studied by Feng (Feng et al., 2015) using WIA. These studies demonstrate that plaque properties could be identified by using arterial waveform, but no evidence has been shown to differentiate between vulnerable and stable plaques.

Recent research findings, based on physical testing of human carotid arterial plaques, indicated that vulnerable plaques may possess unique mechanical properties associated with their specific structures and sub-components (Cunnane et al. 2016; Kobielarz et al. 2020). This finding inspired a potential approach to assess the vulnerability of atherosclerosis non-invasively. Specifically, the clinical significance of arterial pulse waveform to the local physical properties of the cardiovascular system has been recognized for several decades (Xiao et al. 2019). For instance, the pulsatile wave velocity (PWV), which is a function of the stiffness of the arterial wall, has been used as a clinical index to assess cardiovascular disease (Bogatu et al. 2020). Clearly, the behaviour of the arterial waveform interrelates with the components and mechanical properties of the arterial plaque. Hence, we propose that the specific properties of vulnerable plaque would lead to unique arterial blood waveforms, which provides an alternative route to detect vulnerable arterial plaques non-invasively. Most recently, the *in vitro* experiments performed in our research group found that a significant difference in arterial waveforms exists between the arterial system with the soft plaque (thin-cap fibroatheroma) and hard plaque (fibro-calcified) (Abdulsalam and Feng 2019). This finding leads to a

hypothesis that a unique correlation might exist between the arterial waveforms and the composition of the arterial plaque. In this study, a pioneer arterial waveform analysis approach, Non-WIA (Feng 2010), was proposed to distinguish between the various kinds of plaque at an early stage (30% blockage degree) and inform the vulnerability of plaques by defining the relationship between the composition of the arterial plaque and arterial waveforms.

2. Non-invasive wave intensity analysis (Non-WIA)

Wave intensity analysis (WIA) was first introduced by Parker and Jones (1990). The technique is based on the solution of the method of characteristics to the 1D equations of mass and momentum conservation using the simultaneous measurements of pressure and velocity invasively. Invasive WIA has been used in hemodynamic studies throughout the arterial bed (Khir and Parker 2005; Feng et al. 2007). WIA was modified by Feng (2010), referred as non-invasive wave intensity analysis (Non-WIA), and has the advantage to separate arterial waveform into forward and backward components based on the measured diameter of the artery and velocity of blood flow, which allows for WIA to be used in the arterial waveform analysis non-invasively. To date, Non-WIA has been widely used in *in-vivo* cardiovascular mechanics studies, for instance, in the coronary artery, the pulmonary artery and the common carotid artery (Biglino et al. 2012; Quail et al. 2015; Pomella et al. 2017).

The wave separation depends on the value of wave speed, *c*, which is a key parameter of WIA. Wave speed is a function of tube distensibility and fluid density that can be calculated by Eq (1):

$$c = \sqrt{\frac{1}{\rho D_s}} \tag{1}$$

where c is the wave speed, ρ is fluid density and D_s is the tube distensibility. In this study, the wave speed is determined by using *Ln DU–loop* (Feng and Khir 2010), where the infinite small changes of the diameter, along with the propagation of the wavefronts, are linearly proportional to the relevant velocity components.

The separated forward and backward wavefronts can be calculated using Eq (2-3).

$$dD_{\pm} = \frac{1}{2}\left(dD_{\pm} \frac{D}{2c}\, dU\right) \tag{2}$$

$$dU_{\pm} = \frac{1}{2}\left(dU_{\pm} \frac{2c}{D}\, dD\right) \tag{3}$$

where $dD_{\pm}$ and $dU_{\pm}$ are the forward and backward components of the diameter and velocity changes, D is the initial diameter and dD and dU represent the infinite small changes of the measured diameter and velocity. Then, the forward and the backward diameter and velocity can be obtained by the integration of the $dD_{\pm}$ and $dU_{\pm}$ by using Eq (4-5):

$$D_{\pm} = \sum_{t=0}^{T} dD_{\pm} + D_0 \tag{4}$$

$$U_{\pm} = \sum_{t=0}^{T} dU_{\pm} + U_0 \tag{5}$$

where T is the period total time and D_0 and U_0 are the initial value of the diameter and velocity.

Wave intensity (W/m^2) is a typical parameter in the WIA method, which illustrates the energy flux that is transferred by the wave. The net of wave intensity, *dI*, can be obtained by the multiplication of the changes of the measured pressure (*dP*) and the velocity (*dU*). The net of non-invasive wave intensity, $_{n}dI$, can be obtained by the multiplication of the change of diameter and velocity. The separated forward and backward, $_{n}dI_{\pm}$, can be calculated by Eq (6),

$$_{n}dI_{\pm} = \pm \frac{1}{4\,(D/2c)}\left(dD \pm \frac{D}{2c}\, dU\right)^2 \tag{6}$$

3. Methods

In this study, five types of plaques, composed of the main components of the arterial plaques, were fabricated and implemented into an *in-vitro* arterial system. The arterial waveforms including the pressure, velocity and diameter of the arterial wall were measured simultaneously proximal to the plaque position. After five minutes from starting the experiment, the measured data were collected and each plaque was applied three times to ensure reproducibility. The measured waveforms were analysed using Non-WIA so that the reflected wave components could be extracted to illustrate the properties and types of the plaques.

3.1. Artificial plaque preparation

Based on the histological study of the human carotid arterial plaque, Butcovan et al. (2016) classified carotid atherosclerosis into three types: (i) stable plaques including fibroatheroma (FA) and fibro-calcified plaque (FC); (ii) unstable plaques such as plaque rupture (PR), plaque erosion (PE) and calcified nodule (CN); and (iii) vulnerable plaques such as thin-cap fibroatheroma (TCFA). The features of these types of plaques are demonstrated in Table 1. In this study, the artificial plaques with identical characteristics as that of human carotid atherosclerosis, mentioned before, were fabricated manually (Fig. 1).

The following components were used to form the artificial plaques: (i) calcium chloride hexahydrate (Sigma – Aldrich), (ii) Col (type III, Sigma – Aldrich, St. Louis, MO) and (iii) lipid core-soybean oil. Calcium chloride hexahydrate has been widely used in medical research such as composite living fibres for creating tissue constructs (Akbari et al. 2014) and phosphatidylthreonine and lipid-mediated control (Arroyo-olarte et al. 2015). Type III Col was used to study the Col chain in the human placenta in the previous studies (Sage and Bornstein 1979). In this study, this type of Col was used to fabricate the FC and the Col part of the plaque. Soybean oil was used to form the lipid core of the artificial plaques in this study. Gelatine (from bovine skin, type B, Sigma – Aldrich, St. Louis, MO) and Col (from human placenta, type III, Sigma – Aldrich, St. Louis, MO) were mixed using deionized water to fabricate the FC (Guo et al. 2013). The *in vitro* study by Teng (Teng et al. 2014) was referred to for the size and shape of the artificial plaque components. The length of the plaque is 21.5 mm, width 6.5 mm and the blockage degree in all plaques were 30%± 5 %. The examples of the stable, unstable and vulnerable plaques distinguished by various percentages of the plaque components (FC, LC, Ca, Col) are demonstrated in Fig. 1.

3.2. Experimental setup

The *in-vitro* experiment (Fig. 2) consisted of the pulsatile blood pump (Harvard Apparatus, USA) and artificial artery system, formed with latex Penrose tubing (Kent Elastomer, UK). The pulsatile pressure, flow rate and change of the diameter were measured by the tipped catheter pressure sensor (Millar Instruments, TX, USA), transonic flow probes (Transonic System, NY, USA) and sonometric crystals (Sonometric Cooperation, ONT, Canada), respectively.

The Pulsatile Blood Pump (Harvard Apparatus, Holliston, Massachusetts, US) was used to generate the pulsatile arterial waveforms. The shape and amplitude of the arterial waveform, which are similar to those of the human carotid arteries, were determined by the settings of the pump and the compliance of the arterial system. The pump rate was set at 70 RPM and stroke rate at 15cc. The simulated ventricle was connected by polyvinyl chloride

(PVC) tube to receive and pump the circulation fluid. The diameter, flow rate and pressure measurements, via change of the time, were taken simultaneously at 5 mm proximal to the artificial plaque. The distance between artificial plaque, pressure transducer, flow probe and crystals was less than 5mm. The artificial blood vessel diameter was 9.5 mm.

The range of pressure for a healthy condition (no plaque) was between 127 mmHg to 75 mmHg. The housing program, based on Non-WIA and edited using Matlab R 2018a, was used for analysing the data.

Table 1 The compositions of the three types of plaque: stable; unstable and vulnerable plaques (referred to from Butcovan et al. (2016))

Plaque type	Thickness of FC (mm)	LC %	Ca %	Col%
FA (Stable)	0.35	57 %	0 %	43 %
FC (Stable)	0.27	47 %	6.2 %	47 %
TCFA (vulnerable)	0.022	26 %	0 %	74 %
PR (Unstable)	0.017	22 %	0 %	78 %
PE (Unstable)	0.013	71 %	18 %	12 %

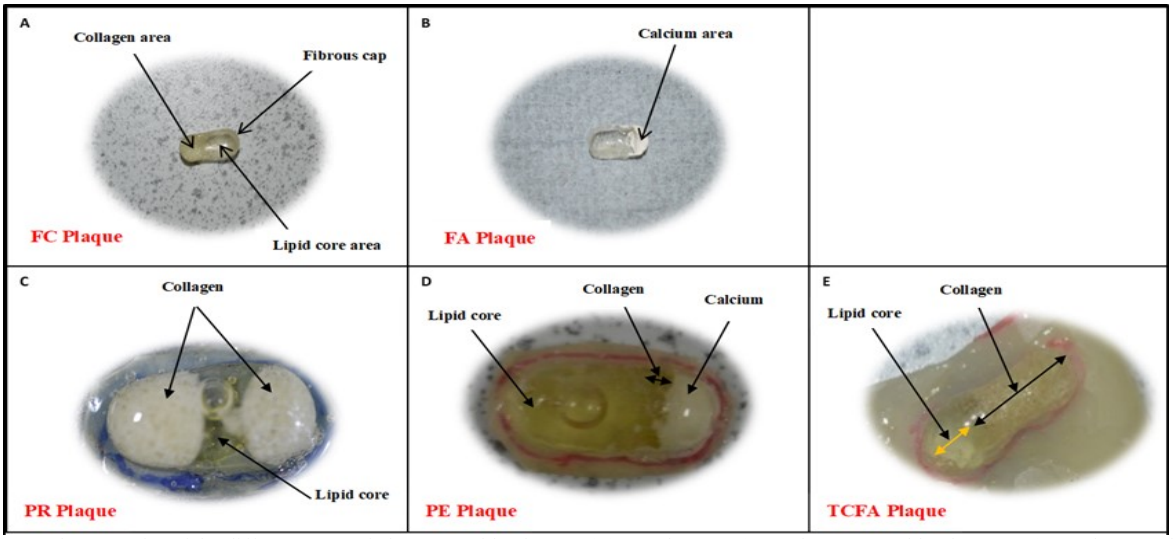

Fig. 1. The examples of the different types of plaques. Stable plaques: (A) FA plaque, (B) FC plaque; Unstable Plaques: (C) PR plaque and (D) PE plaque; Vulnerable Plaque: (E) TCFA plaque.

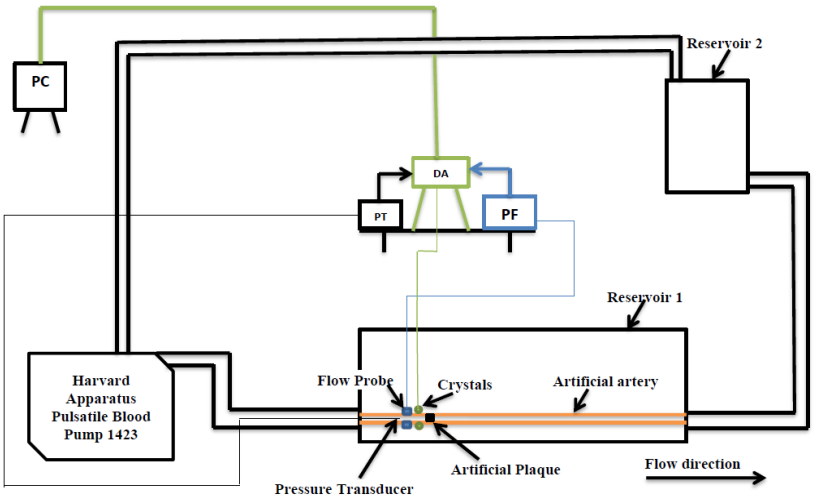

Fig. 2. The experiment setup. PT is pressure transducer bridge amplifier; PF is Perivascular flow meter; DA is data acquisition and PC is personal computer. The measurements: flow probe, pressure transducer and crystals were mounted before the plaque at 26.6 cm from the injection site; and the distance between each of them was less than 5mm. The fluid level was 2 cm above the artificial artery.

4. Results

4.1. The measured diameter and velocity

Fig. 3A shows that the diameter of the tube wall was measured at 5mm proximal to the soft (PE, TCFA) and hard (FC) plaques. The diameter measured at the same position with no plaque implemented (Healthy) is also plotted in the same graph (solid line). In this figure,

the highest amplitude of diameter waveform was observed for the case with FC plaque, which is 4.2% higher than the diameter measured at the same position for a healthy one (Table. 2). The soft plaques also caused a slight increase of the measured diameter waveform by 3.4% for TCFA and 3.9% for PE plaque, respectively. The exceptional results are presented in Fig. 3B and indicate that PR (soft) plaque generated the highest measured diameter amplitude with a 6.7% increase compared to a healthy case and the lowest amplitude of diameter was found with FA plaques.

The measured pulsatile velocity waveforms in the tube with the attached TCFA, FC and PE plaques are exhibited in Fig. 3C. The lowest amplitude of velocity waveform was observed in the system with FC plaque. In comparison with a healthy case, FC plaque caused a 3.8% decrease in peak velocity. In contrast, the velocity waveforms measured in the system with TCFA and PE plaques are similar to those measured in the healthy system. Fig. 3D shows the velocity waveform measured in the system with FA (hard) and PR (soft) plaques and a healthy artery (no plaque). In this figure, the highest velocity waveform was observed with FA (hard) plaque, while the lowest one links with PR plaque (soft). Particularly, PR plaques caused a considerable decrease in the peak velocity in comparison to a healthy scenario (approximately 8% decrease).

4.2. Separated forward and backward diameter

4.2.1. The wave speed determination (no plaque)

Fig. 4 shows the determination of wave speed by using the non-invasive ln DU-loop technique. The artificial artery diameter was 9.5mm, which responded to the single pulse from the pulsatile blood pump. The early systolic period (initial part of the loop) is visibly linear from the beginning of injection. The linearity of the early systolic period means that the forward waves are only existed and the straight line corresponds to $\frac{1}{2}c$. The indicated line, therefore, is corresponded to c = 3.5m/s, and this value was used to separate the measured velocities and diameters of no plaque, FC, TCFA, PE, FA and PR plaques.

4.2.2. The separated waveforms with FC, TCFA and PE plaques

The separation of the diameter for the three types of plaques is illustrated in Fig.5 A–D. Compared with the healthy condition, an increase in the backward diameter was observed for all three types of plaques. FC plaque, with the components of LC (47%), Ca (6%) and Col (47%), causes +39% of increase in diameter (0.61∝ 0.44 mm), whereas TCFA plaque made of LC (26%) and Col (74%), results in 30% of increase in diameter ($0.57 \propto 0.44\ mm$). PE plaque (70% of LC, 18% of Ca and 12% of Col), having a lower content of LC, shows the lowest value of diameter. In comparison with the diameter measured in the healthy condition, PE plaque causes a +23% increase in diameter ($0.55 \propto 0.44\ mm$).

4.2.3. The separated waveform with PR and FA plaques

Fig. 6 presents the results of waveforms associated with the PR and FA plaques. The composition of PR soft plaque in this study, including 22% of LC, 0% of Ca and 78% of Col, generated 0.61mm backward diameter amplitude. In comparison with a healthy condition, PR plaque causes a 57% increase in diameter. The backward diameter of FA plaque, which contained 57% of LC, 0% of Ca and 43% of Col, increased approximately 9% in comparison with a healthy artery.

The results for the measured and separated waveforms related to the PR and FA plaques are inconsistent with our expectations. We expected that FA (hard) plaque caused the stronger reflection, accordingly leading to the higher diameter waveform. Whereas, the lower diameter waveform was expected with PR plaque due to the potentially less strong reflection to be generated by the soft plaque. The phenomena with higher diameter related to PR plaque and the lower diameter linking with FA plaque is opposite to our expectations and it was observed among all of the tests. Therefore, it is speculated that the underpinning mechanism lies in the compositions of the plaques, particularly the percentage of typical components.

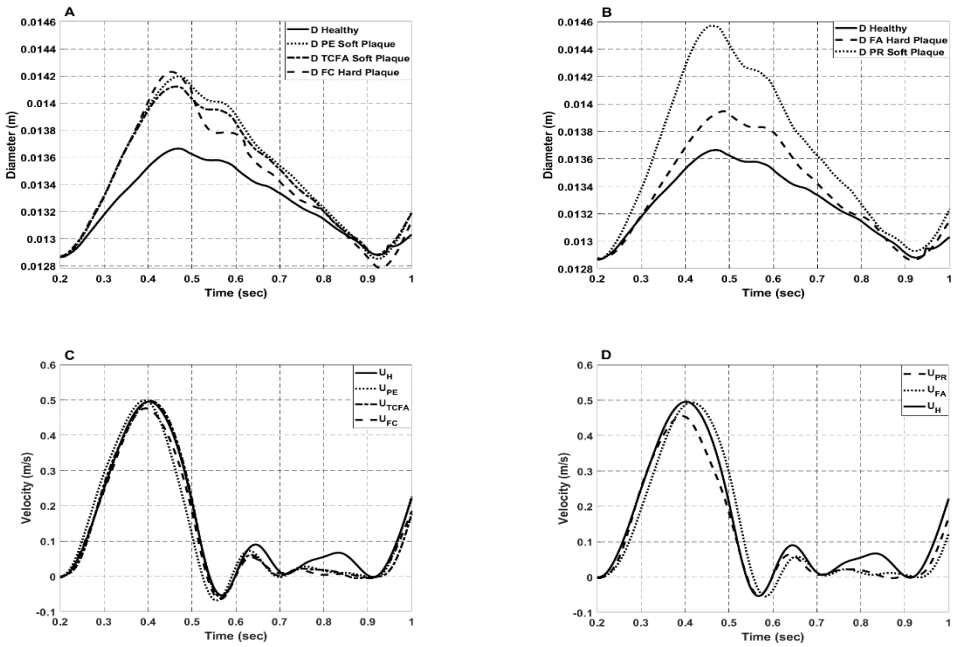

Fig. 3. (A) The measured diameter for hard plaque (FC), soft plaques (PE and TCFA) and healthy case; (B) the measured diameter for hard plaque (FA), soft plaque (PR) and healthy case; (C) the measured velocities of hard plaque (FC), soft plaques (PE and TCFA) and healthy case; (D) the measured velocities of hard plaque (FA), soft plaque (PR) and healthy case.

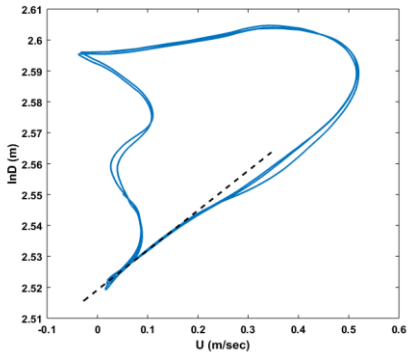

Fig. 4. The wave speed determined by the non-invasive ln DU-loop technique from the experiment. The linear part is visible which corresponds to 0.5c. The value of c is 3.5 m/s, which was used to separate the measured waveform into forward and backward.

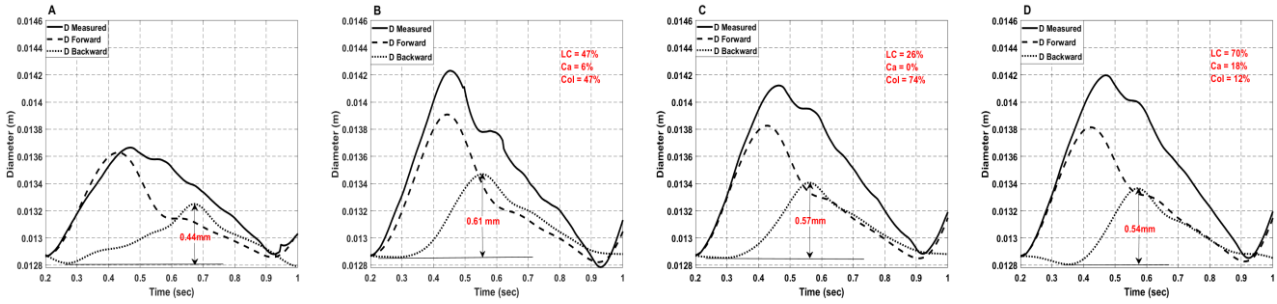

Fig. 5. Measured, forward and backward arterial diameter in the healthy tube and tubes with hard plaque (FC) and soft plaques (TCFA and PE) are illustrated in (A), (B), (C) and (D), respectively. The solid, dashed and dotted lines represent the measured, forward and backward waves, respectively. The effect of FC plaque, which has 47% of LC, 6% of Ca and 47% of Col, generates a higher amplitude of diameter than PE and TCFA plaques do. The diameter of PE plaque is lower than that of TCFA.

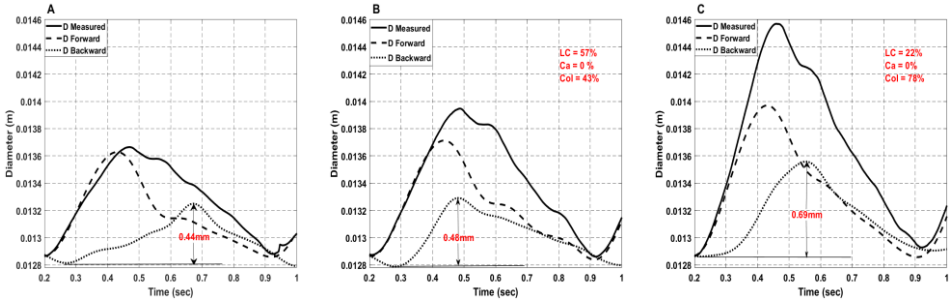

Fig. 6. A, B and C show the diameter waveform separations of no plaque condition, FA hard plaque and PR soft plaque, respectively. The solid, dashed and dotted lines in the three graphs represent the measured, forward and backward waves, respectively. The PR soft plaque generated higher backward diameter amplitude than FA hard plaque.

4.2.4. Statistical analysis results

The results of the correlational analysis between backward diameter amplitude with Col, LC and Ca are displayed in Fig. 7. A strong positive correlation between backward diameter amplitude and Col was observed with R = 0.75 (Fig. 7A). In addition, a strong negative correlation was found between backward diameter and LC with R = 8.2 (Fig. 7B). However, no significant correlation was found between Ca and backward diameter amplitude with R = 0.3 (Fig. 7C). These results indicate that a higher percentage of Col in the plaque is associated with a higher backward diameter, while the higher percentage of LC is related to a lower backward diameter.

5. Discussions

The arterial system in the human body is very complex, particularly in bifurcation, and its properties can change from one place to another. This can cause the reflection, transition and change

of diameter during the cardiac cycle (Parker 2009). The existence of arterial plaque could increase the rate of reflections, which makes the waveform more complex. Therefore, it is very important to understand the wave behaviour in the arterial system to understand its wave properties. This can be done by separating the measured waves into forward and backward waves to extract more information from the measured waveforms. The forward waves are mostly caused by the heart, while the backward waves are from the reflections (Parker 2009).

There has been growing evidence that arterial plaque composition could be characterized by the arterial waveform which may lead to identifying plaque vulnerability (Feng et al. 2015; Li et al. 2018; Abdulsalam and Feng 2019). This study attempted to investigate the effect of plaque composition on arterial waveform using different types of plaques.

One observation of this study is that the measured diameter of stable plaque generated higher amplitude than the unstable plaques, while the measured pulsatile velocity of unstable plaques generated higher amplitude than the stable plaques. For example, FC plaque produced higher amplitude in diameter than PE and TCFA plaques (Fig. 3A). A possible explanation is that the mechanical properties of stable plaque, particularly the arterial plaque stiffness, which includes Ca and Col, are greater than soft plaque. As can be seen from Table 1, the plaque composition of FC plaque contained 47% Col and 6% Ca, PE plaque had 12% Col and 18% Ca and TCFA plaque included 74% Col and 0% Ca. The strong reflection generated by FC led to a higher amplitude in diameter in comparison with PE and TCFA plaques. Meanwhile, a higher amplitude of diameter was observed in the vessels attached to the PR plaque, while a lower amplitude of diameter was observed in the FA plaque (Fig. 3B). This finding is inconsistent with our expectations. Clinically, PR plaque is a soft plaque, while FA is a hard plaque (Narula et al. 2013). It is expected that PR plaque, clinically known as a soft plaque, causes weak reflection and is associated with lower diameter, whereas FA plaque as a hard plaque, leads to a strong reflection, resulting in a higher amplitude of diameter. This non-anticipated result could be attributed to their plaque compositions.

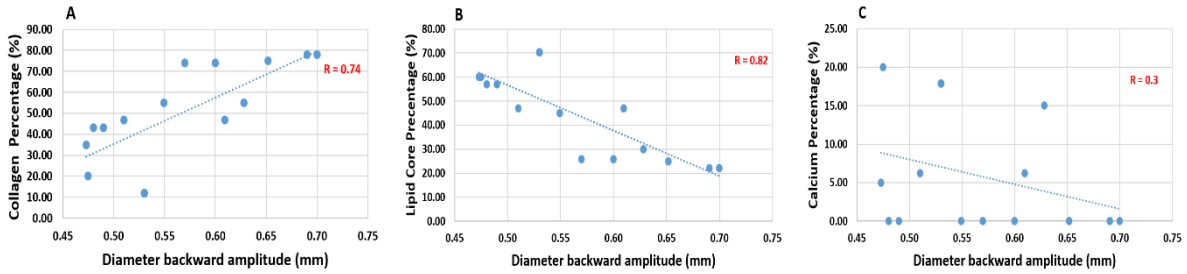

Fig. 7. (A) shows the effect of arterial plaque Col on arterial backward wall movement. This result shows that there is a positive significant correlation between Col and backward diameter amplitude. (B) illustrates that there is a negative correlation between LC and backward diameter amplitude. (C) demonstrates that the effect of Ca in this study on arterial backward diameter had negative correlation.

The separated forward and backward diameters for the stable and vulnerable plaques with the different components are presented in Fig. 5 and Fig. 6. The correlation of the percentages of each type of the components and the amplitude of the backward diameters are plotted in Fig.7. A positive correlation was found between Col and backward diameter amplitude and a negative correlation was found between LC and backward diameter. This implies that the higher percentage of Col in PR plaque caused higher backward diameter amplitude, while the higher percentage of LC in the plaque produced lower backward diameter. Therefore, the plaque compositions, particularly in LC and Col, have a direct effect on the amplitude of backward diameter. The increase of Col in arterial plaque resulted in higher diameter amplitude, while the increase of LC led to a decrease in the backward diameter.

The research findings from this study indicate the possibility of using arterial waveforms to detect the compositions of stable and vulnerable plaques in clinical settings. In the current clinical practice, the narrowing blood vessels are measured by using the ultrasound echo tracking and Doppler system, which allows for the diameter of the arterial wall and velocity of the blood to be measured non-invasively. It has been proved that Non-WIA is a reliable approach to analyse the carotid arterial waveforms measured by using the ultrasound echo and Doppler system (Pomella et al. 2017). Therefore, the approach to detect the compositions of the stable and vulnerable plaques, developed in this study, has potential clinical applications.

As mentioned above, the arterial system in the human body is very complex. In particular, the bifurcations in the arterial system can disturb the blood flow performance around the regions of the bifurcation (Feng 2020) and could inherently alter the propagation pattern of arterial waveforms. Meanwhile, the geometries and properties of the arterial system can change from one place to another, which also have an influence on blood flow performance (Wang 2020) and the nature of arterial waveforms. To the authors' best of knowledge, the effect of compositions of the plaques on the propagation of the arterial waveforms has not been studied in depth. Therefore, the findings from this study provide novel knowledge regarding the nature of the types of plaques related to arterial waveform propagation, providing important information to distinguish between stable, unstable and vulnerable plaques. The findings from this study, combined with the feasibility of using Non-WIA to analyse arterial waveforms in a clinical setting, offer a potential novel medical diagnosis approach to distinguish between the vulnerable and stable plaques.

An *in-vivo* study would be essential to further investigate the correlation between arterial waveforms and the nature of plaques in clinical settings. It is envisaged that the complex of the arterial system in the human body, such as bifurcations, the variation of the stiffness of the local arterial wall, would add more complicated information for the arterial waveforms. Extracting the information of compositions of the plaque accurately and excluding the influence of the other factor(s) would be more challenging. The key information from this *in-vitro* study is the correlation of the backward arterial waveforms (amplitude) and compositions of the different types of plaques. In this study, we consider pulsatile wave velocity (PWV) as a constant due to the uniform vessel used. In the

human body, properties of the arterial wall varied along with the arterial system, therefore, variation of PWV along with arterial system needs to be considered. Furthermore, alteration of the waveform caused by the bifurcations and local arterial wall properties also needs to be considered.

In this study, the uniform outer shape of the artificial plaque was fabricated and employed, which are different from the irregular shape of the real plaques observed in clinical studies. This is also a major limitation of this study. The effect of the irregular shape of the plaques is worth being further investigated in the future. It is also noticed in this study that the characteristic of arterial waveforms is sensitive to the location of the different compositions within the plaques. The effect of the location of the different compositions on the arterial waveforms will be further investigated. Additionally, investigation and experimentation into the effect of Ca with various percentages together with Col are strongly recommended to provide a clearer picture of its effect on arterial backward amplitude. Finally, the severe stenosis degree (between 50 and 90%) should be performed for further investigation about the effect of the combination of the degree of block and plaque composition on the arterial waveform.

6. Conclusion

This study investigates the effect of artificial plaque compositions on the arterial waveform. The results show that plaques with less LC percentage and a higher amount of Col and Ca percentage generate higher backward diameter amplitude. In addition, a strong positive correlation was found between Col percentage and backward diameter amplitude, while a significant negative correlation was observed between LC and backward diameter amplitude. The strong correlation between the compositions of the plaques with the backward waveforms observed in this study demonstrated that the components of the arterial plaques could be distinguished by the arterial waveforms. This finding might lead to a potential novel non-invasive clinical approach to determine the compositions of the plaque and distinguish the stable and vulnerable arterial plaques at the early stage

Acknowledgement

This work is partially supported by the Grant from EPSRC (Engineering and Physical Sciences Research Council) EP/R001901/1. The authors would like to acknowledge the support from the technical team in the Department of Engineering at Manchester Metropolitan University.

References

Abdulsalam, M., Feng, J., 2019. Distinguish the stable and unstable plaques based on arterial waveform analysis. Procedia Structural Integrity. 15, 2–7. https://doi.org/10.1016/j.prostr.2019.07.002

Akbari, M., Tamayol, A., Laforte, V., Annabi, N., Najafabadi, A. H., Khademhosseini, A., Juncker, D., 2014. Composite Living Fibers for Creating Tissue Constructs Using Textile Techniques. J. AFM. 11(24), 4060–4067. https://doi.org/10.1002/adfm.201303655

Ammirati, E., Moroni, F., Norata, G. D., Magnoni, M., Camici, P. G., 2015. Markers of inflammation associated with plaque progression and instability in patients with carotid atherosclerosis. Mediators Inflamm. 2015, 1-15. https://doi.org/10.1155/2015/718329

Arroyo-olarte, R. D., Brouwers, J. F., Kuchipudi, A., Helms, J. B., 2015. Phosphatidylthreonine and Lipid-Mediated Control of Parasite Virulence. PLoS. Biol. 13(11), 1–24. https://doi.org/10.5061/dryad.564sc

Bentzon, J. F., Otsuka, F., Virmani, R., Falk, E., 2014. Mechanisms of plaque formation and rupture. Circ. Res. 114(12), 1852–1866. https://doi.org/10.1161/CIRCRESAHA.114.302721

Biglino, G., Steeden, J. A., Baker, C., Schievano, S., Taylor, A. M., Parker, K. H., 2012. A non-invasive clinical application of wave intensity analysis based on ultrahigh temporal resolution phase-contrast cardiovascular magnetic resonance. J. Cardiovasc. Reson. 14(1), 2–9. https://doi.org/10.1186/1532-429X-14-57

Bogatu, L. I., Turco, S., Mischi, M., Woerlee, P., Bouwman, A., Korsten, E. H. H. M., Muehlsteff, J., 2020. A modelling framework for assessment of arterial compliance by fusion of oscillometry and pulse wave velocity information. Comput Methods Programs Biomed. 196, 105492. https://doi.org/10.1016/j.cmpb.2020.105492

Butcovan, D., Mocanu, V., Baran, D., Ciurescu, D., Tinica, G., 2016. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens. Exp. Ther. Med. 11(5), 2028–2032. https://doi.org/10.3892/etm.2016.3096

Chan, J. M. S., Monaco, C., Wylezinska-Arridge, M., Tremoleda, J. L., Gibbs, R. G. J., 2014. Imaging of the vulnerable carotid plaque: Biological targeting of inflammation in atherosclerosis using iron oxide particles and MRI. Eur J Vasc Endovasc Surg. 47(5), 462–469. https://doi.org/10.1016/j.ejvs.2014.01.017

Cunnane, E. M., Mulvihill, J. J. E., Barrett, H. E., Hennessy, M. M., Kavanagh, E. G., Walsh, M. T., 2016. Mechanical properties and composition of carotid and femoral atherosclerotic plaques: A comparative study. J. Biomech. 49(15), 3697–3704.

Feng, J., Khir, A. W., 2010. Determination of wave speed and wave separation in the arteries using diameter and velocity. J. Biomech. 43(3), 455–462. https://doi.org/10.1016/j.jbiomech.2009.09.046

Feng, J., Long, Q., Khir, A. W. A., 2007. Wave dissipation in flexible tubes in the time domain : In vitro model of arterial waves. J. Biomech. 40, 2130–2138. https://doi.org/10.1016/j.jbiomech.2006.10.034

Feng, J., Rajeswaran, T., He, S., Wilkinson, F. L., Serracino-Inglott, F., Azzawi, M., Alexander, M. Y., 2015. Investigation of the composition of arterial plaques based on arterial waveforms and material properties. Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS. 2015, 993–996. https://doi.org/10.1109/EMBC.2015.7318531

Feng, J., Wang, N., Wang, Y., Tang, X., Yuan, J., 2020. Haemodynamic Mechanism of Formation and Distribution of Coronary Atherosclerosis: A Lesion-Specific Model. Proc. Inst. Mech. Eng H. 234(11), 1187–1196.

Finn, A. V., Nakano, M., Narula, J., Kolodgie, F. D., Virmani, R., 2010. Concept of vulnerable/unstable plaque. Arterioscler. Thromb. Vasc. Biol. 30(7), 1282–1292. https://doi.org/10.1161/ATVBAHA.108.179739

Guo, J. I., Saylor, D. M., Glaser, E. P., Patwardhan, D. V., 2013. Impact of Artificial Plaque Composition on Drug Transport. J. Pharm. Sci. 102(6), 1905–1914. https://doi.org/10.1002/jps

Khir, A. W., Parker, K. H., 2005. Wave intensity in the ascending aorta: Effects of arterial occlusion. J. Biomech. 38(4), 647–655. https://doi.org/10.1016/j.jbiomech.2004.05.039

Kobielarz, M., Kozuń, M., Gąsior-Głogowska, M., Chwiłkowska, A., 2020. Mechanical and structural properties of different types of human aortic atherosclerotic plaques. J. Mech Behav Biomed Mater. 109, 103837. https://doi.org/10.1016/j.jmbbm.2020.103837

Li, R. X., Apostolakis, I. Z., Kemper, P., McGarry, M. D. J., Ip, A., Connolly, E. S., Konofagou, E. E., 2018. Pulse Wave Imaging in Carotid Artery Stenosis Human Patients in Vivo. Ultrasound. Med. Biol. 45(2), 1–14. https://doi.org/10.1016/j.ultrasmedbio.2018.07.013

Libby, P., Ridker, P. M., Hansson, G. K., 2011. Progress and

challenges in translating the biology of atherosclerosis. Nature. 473, 317–325. https://doi.org/10.1038/nature10146

Narula, J., Nakano, M., Virmani, R., Kolodgie, F. D., Petersen, R., Newcomb, R., Finn, A. V., 2013. Histopathologic characteristics of atherosclerotic coronary disease and implications of the findings for the invasive and noninvasive detection of vulnerable plaques. J. Am. Coll. Cardiol. 61(10), 1041–1051. https://doi.org/10.1016/j.jacc.2012.10.054

Nichols, M., Townsend, N., Scarborough, P., Rayner, M., 2013. Cardiovascular disease in Europe : epidemiological update. EUR. HEART. J. (34), 3028–3034. https://doi.org/10.1093/eurheartj/eht356

Parker, K. H., Jones, C. J. H., 1990. Forward and Backward Running Waves in the Arteries : Analysis Using the Method of Characteristics. J. Biomech. Eng. 112, 322–326.

Parker, K. H., 2009. An introduction to wave intensity analysis. Med. Biol. Eng. Comput. 47(2), 175–188. https://doi.org/10.1007/s11517-009-0439-y

Pomella, N., Wilhelm, E. N., Kolyva, C., González-Alonso, J., Rakobowchuk, M., Khir, A. W., (2017a). Common Carotid Artery Diameter, Blood Flow Velocity and Wave Intensity Responses at Rest and during Exercise in Young Healthy Humans: A Reproducibility Study. Ultrasound. Med. Biol. 43(5), 943–957. https://doi.org/10.1016/j.ultrasmedbio.2016.12.018

Quail, M. A., Knight, D. S., Steeden, J. A., Taelman, L., Moledina, S., Taylor, A. M., Muthurangu, V., 2015. Noninvasive pulmonary artery wave intensity analysis in pulmonary hypertension. Am. J. Physiol. Heart. Circ. Physiol. 308(12), H1603–H1611. https://doi.org/10.1152/ajpheart.00480.2014

Sage, H., Bornstein, P., 1979. Characterization of a Novel Collagen Chain in Human Placenta and Its Relation to AB Collagen. Biochemistry. 18(17), 3815–3822.

Stroke Association. 2018. Stroke Association. State of the Nation: Stroke Statistics.

Teng, Z., Zhang, Y., Huang, Y., Feng, J., Yuan, J., Lu, Q., Gillard, J. H., 2014. Material properties of components in human carotid atherosclerotic plaques: A uniaxial extension study. Acta Biomaterialia. 10(12), 5055–5063. https://doi.org/10.1016/j.actbio.2014.09.001

Underhill, H. R., Hatsukami, T. S., Cai, J., Yu, W., DeMarco, J. K., Polissar, N. L., Yuan, C., 2010. A noninvasive imaging approach to assess plaque severity: The carotid atherosclerosis score. Am. J. Neuroradiol. 31(6), 1068–1075. https://doi.org/10.3174/ajnr.A2007.

Van Den Oord, S. C. H., Akkus, Z., Renaud, G., Bosch, J. G., Van Der Steen, A. F. W., Sijbrands, E. J. G., Schinkel, A. F. L., 2014. Assessment of carotid atherosclerosis, intraplaque neovascularization, and plaque ulceration using quantitative contrast-enhanced ultrasound in asymptomatic patients with diabetes mellitus. Eur. Heart J. Cardiovasc. Imaging. 15(11), 1213–1218. https://doi.org/10.1093/ehjci/jeu127

Wang, D., Serracino-Inglott, F., Feng J., 2020. Numerical Simulations of Patient-Specific Models with Multiple Plaques in Human Peripheral Artery: A Fluid-Structure Interaction Analysis. Biomech. Model. Mechanobiol. 20, 255-265. https://doi.org/10.1007/s10237-020-01381-w.

Wasserman, B. A., Wityk, R. J., Trout, H. H., Virmani, R., 2005. Low-grade carotid stenosis: Looking beyond the lumen with MRI. Stroke. 36(11), 2504–2513. https://doi.org/10.1161/01.STR.0000185726.83152.00

Xiao, H., Qi, L., Xu, L., Li, D., Hu, B., Zhao, P., Huang, J., 2019. Estimation of wave reflection in aorta from radial pulse waveform by artificial neural network: a numerical study. Comput Methods Programs Biomed. 182, 1-10. https://doi.org/10.1016/j.cmpb.2019.105064

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jiling Feng 

Mohamed Abdulsalam 

Author Statement

We submit a revised original research article entitled “The Compositions of the Vulnerable Plaque and the Effect on Arterial Waveforms” for consideration by Journal of the Mechanical Behavior of Biomedical Materials. We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Authors Contributions:

Mohamed Abdulsalam is a PhD student who conducts the experimental and analysis work. Mohamed drafted the manuscript.
Dr Jiling Feng is a supervisor, responsible for the initializing of research ideas, planning of the project, providing the resources of research, reviewing and editing of the manuscript.