

IMPACT OF FORMALDEHYDE ON MECHANICAL PROPERTIES OF ATHEROSCLEROTIC CAROTID ARTERIES

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Abstract: *Atherosclerosis is a life threatening cardiovascular disease causing lipoprotein accumulation within intima layer of artery wall and thus its thickening. Later stages are characterized by plaque formation with a lipid core separated from lumen by fibrous cap. In case of carotid arteries (CA), rupture of the plaque often results in a stroke. Detailed understanding of mechanical properties of atherosclerotic arteries and their components is essential for improvement of computer models used for prediction of plaque rupture. Samples of atherosclerotic CA from carotid endarterectomy were kept either in saline solution or in formaldehyde solution and then underwent uniaxial tensile testing in two directions. Statistical analysis of the obtained stress-strain responses shows that formaldehyde treatment of the tissue increases significantly the mean stiffness and reduces the dispersion.*

Keywords: Atherosclerotic plaque, Carotid artery, Mechanical properties, Uniaxial tensile test.

1. Introduction

Atherosclerosis is a progressive cardiovascular disease characterized by accumulation of lipoproteins in the inner artery wall layer – intima. Over time the lipoproteins stacked within the intima create a lesion which consists of a lipid core (LC) covered by fibrous tissue forming a new layer – fibrous cap (FC). As the disease progresses, the lesion enlarges and limits or even blocks the blood flow. The most frequent complications are detachment of a part of the lesion or rupture of the FC leading to a release of the lipid into the circulatory system and subsequent formation of blood clots. In case of common or internal CA it results in a stroke with high mortality of the patients (Fisher et al., 2005).

During this process, new components such as fibrous tissue and, in later stages, calcifications are formed within the artery wall. The layered structure of healthy artery wall is known to be heterogeneous and so are even more the specific components of atherosclerotic arteries. Hence, their mechanical properties are highly varying within the affected area (Mulvihill et al., 2013).

An arterial tissue damage occurs when stresses induced by the blood pressure loading exceed the tissue strength. The lipid core is separated from lumen by the fibrous cap, therefore detailed knowledge of mechanical properties of the fibrous cap together with the other components of the atherosclerotic plaque is fundamental for predictions of the plaque rupture risk.

Uniaxial and especially biaxial mechanical tests can help us to understand better the non-linear anisotropic mechanical behaviour of atherosclerotic tissues. Recently, biaxial tests on CAs were presented (Kamenskiy et al., 2011), (Kamenskiy et al., 2014), (Sommer et al., 2009). However, the severity of atherosclerosis was

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much lower, it varied from low to medium. To our best knowledge, there are no published experimental results of biaxial testing of highly atherosclerotic arteries. In contrast, uniaxial tests were realized even with specific tissue components separated from the atheroma and the adjacent arterial wall (Holzapfel et al., 2004). Specimens studied in this paper originated from standard endarterectomy and due to severely diseased intima were also tested in uniaxial tension only. Some of the specimens were fixed in formaldehyde; as this is a standard procedure in histological analyses, the question was whether it impacts on mechanical properties of the tissue substantially.

2. Methods

2.1. Specimen extraction and preparation

All samples were acquired (with an informed consent of the patients) during endarterectomies in St. Anne's University Hospital in Brno from April to December 2019. They consisted of intima layer with atherosclerotic plaque (atheroma) and, in majority of cases, of bifurcation and part of the internal and external CA. They were tested either directly after surgery (within 24 hours), or later, after having been kept frozen at $-18\text{ }^{\circ}\text{C}$ in physiological solution, or after having been treated in formaldehyde (10% solution) for 24 hours. As it was previously shown that freezing does not influence mechanical properties of arterial tissues significantly once the specimen is unfrozen (Ebenstein et al., 2009), (O'Leary et al., 2014), we considered them in the same group as the fresh specimens.

On the day of testing, the samples were submerged in physiological solution and heated slowly to $37\text{ }^{\circ}\text{C}$. For each sample, presence of calcifications was evaluated. Although a standard classification (Stary, 2000) dividing atherosclerosis into 8 categories could not be used without further histological examination, presence of calcifications in most specimens indicated the lesion type VII+. To consider also the tissue anisotropy, specimens were cut off in both axial and circumferential directions; their orientation was decided primarily regarding the tissue damage. Generally, the samples were cut through the lipid core in longitudinal direction. Although the effort was to obtain the same quantity of axial and circumferential specimens, the axial incision through the sample as well as their small size caused the majority of specimens to be circumferential. In total, 70 specimens from 27 patients (18 men, 9 women) were tested with an average age of 69 years; 44 of the specimens were kept in saline (0.9% NaCl) solution and 26 specimens treated with formaldehyde.

If feasible, the fibrous cap was carefully peeled off and tested separately from the intima. However, this was possible only for few specimens ($n = 3$). In majority of cases, efforts of removing the FC lead to tearing the intima. Then the lipid core and calcifications were removed. For several specimens, the removal of calcifications was not possible, because calcifications were firmly fixated in the intima, so their removal caused intima to tear. The specimens were cut from sample using scalpel or, if possible, a dogbone shaped specimen was cut using a special cutting knife. In case of using scalpel, we aimed to obtain a rectangular specimen of 2 mm width.

For each specimen, information about the location within the sample, extend of calcifications and other significant factors were gathered and recorded. The thickness was measured at 3 different locations using distance indicator with 0.01 mm accuracy and the mean value of thickness was used for further analyses. During the specimen preparation, the rest of samples with other specimens were also kept in the heated physiological solution to prevent tissue drying. Directly before testing, 4 markers were made at the surface of the specimen using alcohol based or permanent marker.

2.2. Testing and evaluation

A tailored computer controlled tensile testing device (Camea s.r.o., CZ) was used to perform uniaxial tests. Two clamps were used to grip the specimen which were submerged during the test in physiological solution heated at $37 \pm 0.5\text{ }^{\circ}\text{C}$. Deformation was evaluated off line from positions of the markers on the specimen surface captured with a CCD camera while the force was measured by an electric resistance force sensor.

Pre-tension of 0.02 N was applied to flatten the specimen (for some of the formaldehyde fixed specimens it was higher, up to 0.08 N) and uniaxial tensile test was executed until tear of the specimen. Although both longitudinal and transversal strains can be determined from four markers using software Tibixus, only the

longitudinal strain was used in the calculations. Tissue incompressibility was assumed to calculate the Cauchy stress using the following equation:

$$\sigma = \lambda \frac{F}{bt} \quad (1)$$

where F is the measured force, and b , t and λ represent width, thickness, and longitudinal stretch of the specimen, respectively.

3. Results

Each stress-strain curve was smoothed using moving average and interpolated with cubic B-spline for further analysis. Separately in both groups, which were found similar in sex and age, the curves were averaged through strains at different levels of stresses stepped by 10 kPa. All the results were checked for normality of distribution. The null hypothesis that there is no difference in strains between both groups was tested at all the stress levels using Welch's test due to non-equal variances in both groups. The null hypothesis was rejected in all cases with $p < 0.05$. Strength was not evaluated quantitatively due to the fact that in most cases ($n = 51$) the tear occurred at clamps. Comparison of the results in both group is presented in Fig. 1.

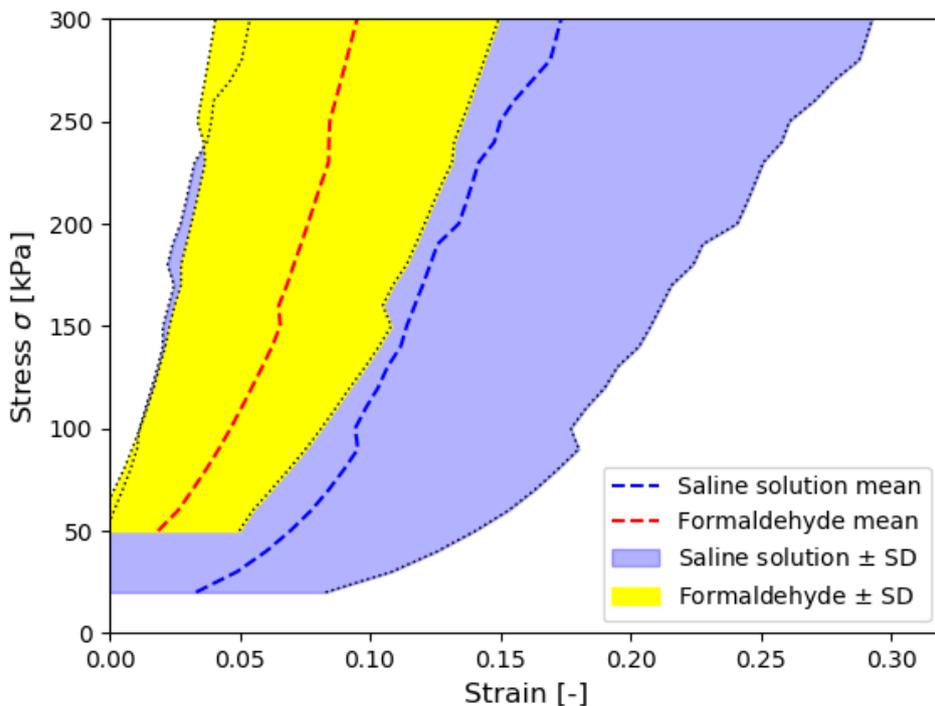


Fig. 1: Uniaxial tensile test results – mean \pm SD.

4. Discussion

Knowledge of mechanical properties of specific components of atherosclerotic CA is fundamental for computational prediction of plaque rupture. In this study, the tests were performed with the extracted atheroma. The specimen gripping was realized with two 2 mm wide spring clamps, similar to clamps used in the previous research (Mulvihill, 2013). Other experiments performed with artery tissues used hooks or jaws to grip the specimens (Kamenskiy et al., 2011), (Holzapfel et al., 2004).

The acquired results confirm a substantial nonlinearity of the tissue response characterized by an increase of stiffness with increasing strain and caused by straightened collagen fibres. This phenomenon is well known for more than 60 years (Roach et. al., 1957) and was confirmed in various studies regarding CAs (Sommer et. Al., 2009), (Gupta et. al., 1997), (Kural et. al., 2012). The same tendency was evident in our experiments, while their direction dependence was not unique. Although generally the circumferential direction is stiffer in arteries due to distribution of collagen fibres (Polzer et al., 2015), in diseased arteries

the directional dependencies may be much more complex as observed in study with biaxial tensile tests of CA (Kamenskiy et al., 2011).

The measured data showed a very large dispersion in stiffness, also reported in other papers (Akyildiz et al., 2014) which, however, was substantially lower in the formaldehyde treated group. Further statistical analysis should be made to determinate possible correlations between the stress-strain response and some factors related to the origin of the tissue. To seek for such factors, we can exploit the stored data about gender, age of patients, extend of calcifications and location of the specimen within the plaque. If these correlations were found and the factors were achievable *in vivo*, they could help us to reduce the dispersion of material data and enhance thus the quality of biomechanical computational models.

5. Conclusion

The realized uniaxial tests of atherosclerotic carotid arteries tissue have shown a significant impact of formaldehyde treatment on their mechanical properties: it increases the initial stiffness and reduces the dispersion of the results compared to fresh specimens. In contrast to results of formaldehyde treated specimens, the data obtained with fresh specimens show a huge variance and indicate existence of 3 stiffness groups. As the specimens originate from various patients and locations within the atherosclerotic plaque, there might be dependencies on some specific factors obtainable *in vivo* (sex, age, direction, calcification, etc.); to identify them, a statistical analysis of a larger cohort of specimens is needed.

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