



Article

Development and approbation of a mobile test bench for mechanical uniaxial compression testing of biological tissues

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Abstract. A technique and a prototype of a mobile test bench for conducting experiments on uniaxial compression of biological tissue samples have been developed. The test bench consists of high-precision scales, an electronic caliper with modified grips, and a video camera. With the help of the test bench, a series of experiments (120 in total) was carried out to determine Young's modulus of atherosclerotic plaques and vascular walls removed from the human body no later than a few hours. A database of plaques and artery walls' mechanical characteristics, as close as possible to their real strength properties, has been formed. In addition, regression dependencies linking Hounsfield units and Young's moduli of atherosclerotic plaques were constructed. The uniaxial compression technique has been verified on the Instron 3342 universal testing machine. Also, to demonstrate the applicability of the developed technique and test bench for uniaxial compression of hard tissues, experiments were conducted with 14 samples of bovine spongy bone.

Keywords: biomechanics, atherosclerosis, mobile test bench, compression, Young's modulus

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Научная статья

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Разработка и апробация мобильного стенда для механических испытаний на одноосное сжатие биологических тканей

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Аннотация. Разработаны методика и прототип мобильного испытательного стенда для проведения экспериментов на одноосное сжатие образцов биологических тканей. Стенд состоит из высокоточных весов, электронного штангенциркуля с модифицированными захватами и видеокамеры. С помощью стенда проведена серия экспериментов (в общей сложности 120) по определению модуля Юнга атеросклеротических бляшек и сосудистых стенок, удаленных из организма не позднее нескольких часов. Сформирована база данных механических характеристик бляшек и стенок артерий, максимально приближенных к их реальным прочностным



свойствам. Кроме того, были построены регрессионные зависимости, связывающие единицы Хаунсфилда и модули Юнга атеросклеротических бляшек. Методика одноосного сжатия верифицирована на универсальной испытательной машине Instron 3342. Также для демонстрации применимости разработанной методики и стенда для одноосного сжатия твердых тканей проведены эксперименты с 14 образцами губчатой кости крупного рогатого скота.

Ключевые слова: биомеханика, атеросклероз, мобильный испытательный стенд, компрессия, модуль Юнга

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Introduction

During biomechanical modeling of arteries affected by atherosclerosis, the problem of determining the mechanical characteristics of vessel walls and atherosclerotic plaques arises [1, 2]. It is also obvious that in the area of atherosclerotic plaques, the wall is pathologically changed, which means that its properties can differ significantly from the ones of healthy tissue. The literature often presents data obtained for plaques as a whole without information on individual structural elements such as core, fibrous cap, or soft component [3]. There are a number of articles devoted to the mechanical properties of arterial wall structural elements (intima, media, and adventitia) and plaques [4], but those studies are carried out on cadaveric samples after a considerable period after death. In addition, testing machines are mostly located in special laboratories outside medical institutions, so tissue samples are stored for quite a long time and often even frozen during transportation to the place of experiments [4]. All this significantly affects the properties of tissues, so the results may differ from the real ones. Thus, the problem of developing mobile testing devices [5, 6], that can be installed directly in the clinic for testing immediately after surgery is an urgent one.

For bone tissues, descriptions of methods for determining mechanical characteristics by computed tomography (CT) based on the Hounsfield units have long been found in the literature [7, 8]. The task seems to be relevant to similarly determine properties of atherosclerotic plaques, which are also identified on CT [9].

The purpose of this work is to create and test the methodology and a prototype of the test bench for conducting experiments on compression of biological tissue samples.



Materials and methods

During the experiment, the movable traverse of the testing machine presses on a sample, and the force and displacement of the traverse are recorded at every moment. The sample's cross-sectional area, as well as its initial height, is considered to be known, therefore, after the experiment, the force at every moment is converted into stress, and the displacement is converted into strain. Further, according to the constructed "stress-strain" dependence, Young's modulus of the sample can be determined. This method for compression testing can be implemented both on a mobile device based on a strain gauge and a linear actuator [6], as well as using high-precision scales and an electronic caliper. In this study, the latter option was implemented with some design modifications.

The test bench for implementing the methodology consists of high-precision scales (measurement accuracy up to 0.01 g, maximum value — 5 kg), an electronic caliper (measurement accuracy up to 0.01 mm), and a video camera (Fig. 1, *a*). For tighter fixation of the caliper and ensuring uniform pressure on the sample, its design was supplemented with removable platforms grown on a 3D printer (Fig. 1, *b*).

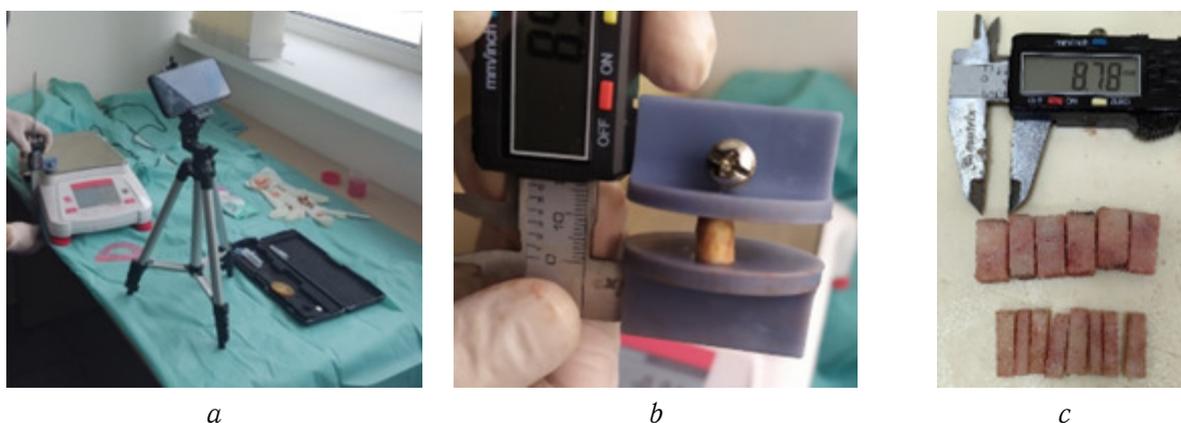


Fig. 1. The test bench components and typical samples: *a* is the general view; *b* are special removable platforms for caliper and a cylindrical sample of the plaque; *c* are samples of bovine cancellous bone (color online)

For experiments, samples of the correct geometric shape with a circle- or square-shaped cross section were prepared from vessels with atherosclerotic lesions. For compression tests, it is necessary to prepare specimens whose linear dimension along the compression axis is greater than any linear dimension of the cross-section. With large lesions, this is not difficult to perform the above for both soft and hard plaques. For example, Fig. 1, *b* shows a cylindrical sample from a femoral artery section (soft plaque). As a rule, the size in the direction of the compression axis ranged from 4.5 to 9 mm, while the linear size of the cross-section (diameter or rib length) varied from 2 to 5 mm. From one side of the vessel belonging to a particular patient with a large amount of atherosclerotic deposits, it may be possible to prepare several samples for testing.

In a number of experiments, it is possible to isolate the plaque fibrous cap: in fact, it is an altered and compacted intima [10, 11]. Usually, a fibrous cap is easily separated from the atherosclerotic deposits in cases of severe calcification. Arterial walls' sections were studied too: both pathologically altered and healthy. When compressing the walls and plaques fibrous caps, as a rule, it was not possible to maintain the required ratio of the geometric parameters of the sample, since the thickness of these structures ranged



from 0.3 to 2.5 mm. But calculations in such cases were also carried out according to the formulas for linear uniaxial compression within the framework of the theory of elasticity. In most experiments, it is possible to preliminarily assess whether the plaque contains a calcified component. Thus, even at the preparation stage, a preliminary classification of the samples was carried out, and for hard plaques, the ultimate strength was additionally determined.

To assess the possibility of using the created prototype to study the strength properties of solid biological tissues, experiments were carried out on uniaxial compression of bovine cancellous bone samples. To do this, prismatic samples of the rectangular cross-section were isolated from the bovine femur metaphysis [12] (Fig. 1, c), the ratio of height to width of which was about 4 to 1.

After preparing the samples, the surface area and the initial height of each of them are measured, that is, the linear size in the direction of the compression axis. Measurements were taken with a digital caliper.

The experiment itself on a mobile test bench is as follows. The sample is placed on the scale's surface, and one caliper jaw with a wide flat nozzle (platform) is brought to its upper face. With the second jaw, the tool rests against the lower edge of the table directly under the scales so that the bar is perpendicular to the plane of the scale platform. The scale and caliper readings are reset to zero. Video recording starts and the operator slowly and evenly moves the upper jaw of the caliper towards the scale's platform, compressing the sample. When the limit value on the scales is reached, a typical sound of sample destruction occurs, or if further compression is impossible (in the case of a high degree of plaque calcification), the experiment stops, as the video recording.

Next, the video is analyzed frame by frame, and the readings of the scales and calipers corresponding to each other are recorded in the table. Further, in the Excel software, the caliper readings are converted into strain, which in this case represents the relative shortening of the sample in the direction of the compression axis. The scale readings are converted into stress. Based on the obtained set of points, a stress-strain dependence is constructed. Further, using the linear section of the graph, Young's modulus can be calculated using the classical formula of Hooke's law for the case of uniaxial tension:

$$E = \sigma/\varepsilon.$$

Young's modulus is calculated at several points (usually 10 points on the graph), after which the average value is taken. This value is taken as the modulus of elasticity for the test sample.

In the case when during the experiment there was a fracture of the calcified plaque, identified by a typical sound, the ultimate strength was additionally determined.

The methodology was verified through mechanical experiments on the Instron 3342 Universal Testing Machine and the mobile test bench on hard (highly calcified) and soft plaque specimens. To verify samples of each type, 7 experiments were carried out. Initially, tests were carried out on a mobile test bench, and the load on the sample was given in the range from 0 to 10 N. This made it possible to deform each sample minimally and not to leave the elastic deformation zone.

Results

In cooperation with the Russian Scientific Center for Radiology and Surgical Technologies named after Academician A. M. Granov, a number of experiments were



conducted aimed at studying mechanical characteristics of atherosclerotic plaques of different densities and localization. Young’s modulus values were obtained for hard (heavily calcified) and soft plaques.

Except for plaques, sections of internal carotid arteries (ICA) and common carotid arteries (CCA), as well as the walls of femoral arteries (FA), which are not subject to changes, were tested on a mobile test bench. In addition, in some cases, it was possible to isolate fibrous caps of the ICA plaques and sections of the walls behind the plaque (the outer part of the vessel behind the calcified plaque, ICA only), for which Young’s moduli were also obtained. Young’s moduli and ultimate strengths of bovine cancellous bone samples were also determined.

The results of the tests with the number of samples were summarized in the Table. The results for each type of tissue were checked using the Kolmogorov – Smirnov test to see if the distribution was normal. In all cases, the distributions were found to be normal, so standard deviations are listed in the table.

Table

Number of samples, average Young’s moduli, and ultimate strengths

| Sample type | Quantity, pcs. | Average Young’s modulus, MPa | Ultimate strength, MPa |
|---------------------------------|----------------|------------------------------|------------------------|
| Soft plaque, ICA | 39 | 0.29±0.17 | – |
| Hard plaque, ICA | 24 | 0.85±0.39 | 1.12±0.67 |
| Soft plaque, FA | 8 | 0.28±0.19 | – |
| Hard plaque, FA | 8 | 1.12±0.54 | 2.24±1.17 |
| Arterial wall, ICA | 14 | 0.32±0.24 | – |
| Arterial wall, CCA | 5 | 0.35±0.24 | – |
| Arterial wall, FA | 10 | 0.27±0.16 | – |
| Fibrous cap | 5 | 0.12±0.05 | – |
| Arterial wall (ICA) near plaque | 7 | 0.08±0.02 | – |
| Bovine cancellous bone | 14 | 318.5±92.7 | 9.25±5.82 |

In addition, regression dependences were built between the Hounsfield units and Young’s moduli obtained during the experiments. For 11 patients at the time of writing, CT scans were obtained with contrasting sections of the vascular bed affected by atherosclerosis (CT scans were available only for patients with plaques in the ICA). On CT, Hounsfield units were calculated for calcified plaques as well as for soft plaques for each patient. Next, a graph of the dependence of Young’s moduli on the Hounsfield units was constructed for each case considered. According to this graph, a regression dependence was built, shown in Fig. 2.

It should be noted that Fig. 2 clearly shows the division into separate groups of soft and hard (calcified) plaques.

It should be noted that the average value of Hounsfield units for plaques was 242. At the same time, in [9] the authors obtained

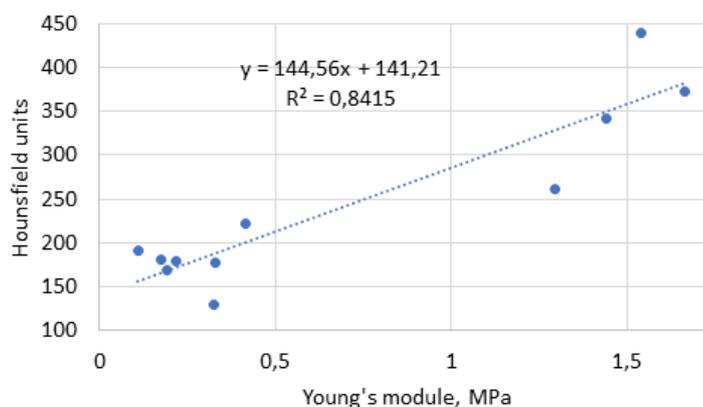


Fig. 2. Dependence between Young’s moduli and Hounsfield units



an average value of 256.7 for the calcified part of the plaque. It is obvious that only dense structural elements of atherosclerotic deposits are clearly visible on CT scans; therefore, the obtained values, even for conditionally soft plaques, were also considered in this comparative analysis.

The results of experiments on the mobile test bench and Instron testing machine for soft plaques obtained during the verification of the method differed by no more than 4.3%, and for hard (heavily calcified) plaques, by no more than 9.5%. Typical stress-strain curves for hard and soft plaques obtained on the Instron testing machine and the mobile test bench are shown in Fig. 3.

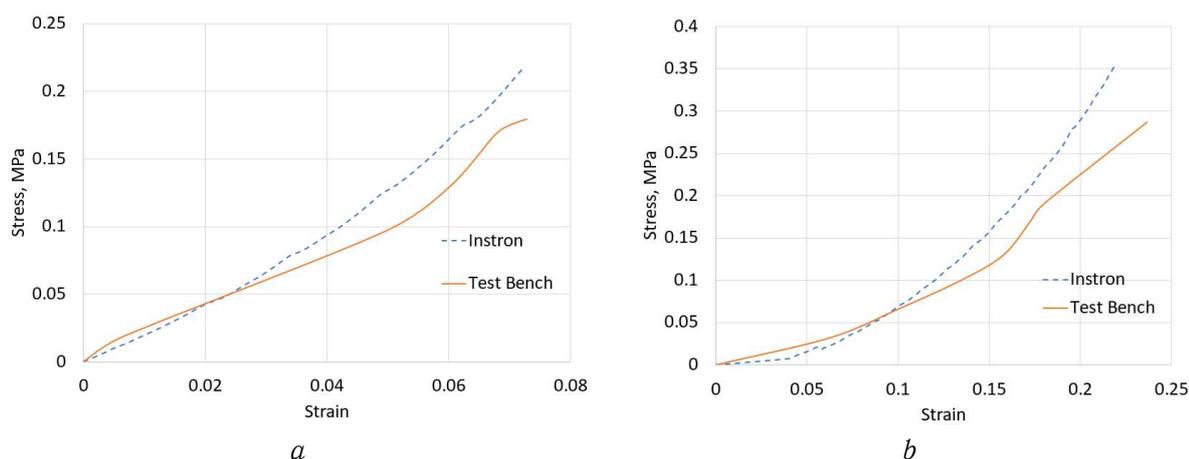


Fig. 3. Typical “stress-strain” dependences obtained on a testing machine and a mobile test bench: *a* — for hard plaques; *b* — for soft plaques

Discussion

A series of experiments were carried out to determine the mechanical characteristics of blood vessels and atherosclerotic plaques immediately after surgical removal, as well as Young’s modulus of bovine cancellous bone. Samples of the studied soft tissues practically did not lose their properties, which allows us to say that numerical calculations using obtained modules will be as accurate and physiological as possible. In fact, this situation, when it is possible to perform experiments with samples immediately after they have been removed from the body, is unique, since they usually need to be transported from the clinic to a mechanical laboratory, which can take quite a long time. In addition, transportation usually involves freezing samples, which can significantly affect their mechanical properties. At the same time, cadaveric material in the framework of experiments does not allow for obtaining reliable information about the properties, which, moreover, has a number of legal restrictions.

A series of experiments were carried out to determine Young’s modulus of atherosclerotic plaques, sections of the vascular wall removed from the body no later than a few hours ago (in some cases experiments were carried out immediately after the surgery), as well as bovine cancellous bone. This made it possible to collect a database of the most relevant mechanical characteristics of plaques and confirm the possibility of using the test bench on bone tissues.

The results are similar to literature data [3, 12–14]. In [13] the average values of the fibrous cap Young’s modulus were 0.082 ± 0.033 MPa (8 samples), which is consistent with the data obtained in this study (0.12 ± 0.05 MPa). Results in [13, 14] are also in good agreement with the data on the properties of the altered ICA wall in the plaque



area. Thus, in [14] the Young's modulus of the altered wall is given as 0.059 ± 0.047 MPa, and the data from the Table (0.08 ± 0.02 MPa) are included in this range of values.

According to the literature data, Young's moduli of ICA walls differ significantly, however, the values from the Table are in excellent order of magnitude agreement with the published values. The mechanical properties of FA are also consistent with literature data. For example, in [15] the Young's modulus of CCA wall is indicated as equal to 0.49 MPa, which is in good agreement with the data from the Table. However, it should be noted that in many modern studies, it is customary to model the FA using hyperelastic material models [16], therefore, to calculate the Mooney – Rivlin constants it will be necessary to build a stress-strain curve using the points obtained on a mobile test bench.

Paper [3] presents the results of the mechanical properties study of carotid and femoral atherosclerotic plaques, and the values obtained in this work differ slightly from the source. In [3] elasticity moduli of plaques in common femoral (0.44 MPa) and carotid (0.89 MPa) arteries differed by almost two times. At the same time, according to data from the Table, there is no such difference for soft and hard plaques in corresponding arteries: 0.28 MPa to 0.29 MPa for soft plaques in femoral and carotid arteries, respectively; 1.12 MPa to 0.85 MPa for hard plaques in femoral and carotid arteries, respectively. Such a difference in ratios may be due to the degree of calcification of the studied plaques in [3] and in this work. Moreover, authors in [3] pointed out that before testing, samples were frozen at a temperature of -20 degrees Celsius, which could change their mechanical properties.

In [17], a value of 2.49 MPa was given for the ultimate strength of a calcified plaque located in the iliac artery, which is quite close to 2.24 MPa obtained for plaques in FA. Anatomically, the femoral artery is, in fact, a continuation of the iliac artery, and atherosclerotic plaques in these vessels often cover fairly long areas, so it seems appropriate to compare mechanical properties with the results from the Table. For the ultimate strength of hard plaques from carotid arteries, we were unable to find data, so this result is still seen as new and requires clarification by enlarging the number of experiments.

As for the calculated Young's moduli of the bovine cancellous bone, the average value of which (318.5 ± 92.7 MPa) corresponds to the literature data [12], where the Young's modulus of femoral metaphysis ranges from 314 to 504 MPa. The calculated ultimate strength of cancellous bone is in good agreement with the ultimate strength of a similar horse bone presented in [18].

Thus, the methodology and its implementation on a mobile test bench show good agreement with literature data for both soft and hard biological tissues.

The human factor proves to be the main limitation of the developed technique. That is the operator's ability to accurately place and then compress the specimen relatively evenly and slowly.

Conclusion

In this study methodology and prototype of a test bench for conducting uniaxial compression experiments on biological tissue samples were developed and tested. A series of experiments was performed to determine the Young's modulus of atherosclerotic plaques and vascular wall sections removed from the body no later than a few hours ago, as well as the Young's modulus of bovine cancellous bone tissue. This made it possible to create a database of the most relevant mechanical characteristics of the plaques. Also, regression dependencies were constructed, connecting Hounsfield units and Young's moduli obtained during the experiments.



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