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General principles of bone tissue testing

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Five basic principles that govern the structure and biomechanical behaviour of biological tissue have been analysed. Many different factors of the mechanical and biological origin, which can significantly affect the mechanical properties and, consequently, the mechanical behaviour of the bone tissue, are presented and discussed. The effect of structural levels in the bone tissue on the selection of proper size of the test specimens is emphasised. Different aspects of the testing procedure of bone tissue are discussed. Peculiarities of the distribution of its biomechanical properties in the man left tibia are described.

Keywords: bone tissue, mechanical properties, testing procedures

1. Introduction

Different mechanical and biological factors have to be taken into account in the investigation of the mechanical behaviour of compact bone tissue.

The mechanical factors that may affect its biomechanical behaviour are the following:

- the type of testing,
- the loading rate or the deformation rate or the strain rate,
- the frequency of scanning impulse or vibration in non-destructive testing,
- the duration of loading,
- the orientation of sample with respect to the axis of material elastic symmetry,
- the shape and size of sample,
- the conditions of the surface processing of samples,
- the temperature and moisture of both the sample and the surrounding environment during an experiment.

In fact, almost all of them have to be taken into account in testing each structural material.

The biological factors are specific to each biological tissue including the bone tissue. Among these factors we can mention the following:

- the race, sex and age of a person,

- the degree of the activity of physiological functions in biological tissue,
- a form and degree of pathological changes in tissue,
- the duration and conditions of storage of both the bone tissue and the specimens made of them until the experiment,
- the localization of bone in the body and the bone tissue samples in the corresponding bone.

Only the knowledge of a whole complex of these factors gives a possibility for reliable evaluation of experimental data and their comparability with data of other investigators. In addition, a separate factor itself may be considered as an independent variable, e.g. the change of the mechanical properties of bone tissue as a function of direction of loading, the age, the strain rate, etc.

2. General principles of the structure and biomechanical behaviour

There are many investigations of structure, biochemical composition and biomechanical properties of different biological tissues. Their complex analysis allows us to postulate five general principles (regularities) determining the structure and biomechanical behaviour of these tissue including the bone tissue. They are the following:

- principle of hierarchy,
- principle of helicality,
- principle of feedback,
- principle of universality,
- principle of optimum.

The *principle of hierarchy* postulates the multilevel structure of biocomposites. It means that in the structure of biological tissue the elementary structural units may be marked out from which the larger structural elements are formed. These larger elements, in turn, form the new larger structural elements, etc. On each structural level a biological tissue may be considered as a composite material.

The *principle of helicality* postulates that biological tissue has the spatial structure. It means that in the biological tissue there exist different twisted (helical) configurations of the structural components including also their corrugated and crimped configurations. More or less distinct helicality may be seen on each structural level. Helicality of structural components is found especially in soft biological tissuess, which are generally subjected to the tensile loading during physiological processes.

The *principle of feedback* involves the adaptation of biological tissue to the mechanical loads and determines the remodelling process. As a result of applied load the intensity of physiological processes in the biological tissue changes. It causes also the slow changes of material structure in such a way that provides an effective resistance of biological tissue to given loading condition. It is one of the most significant distinctions between alive and non-alive materials.

The *principle of universality* postulates the existence of different reinforcement forms at one and the same time. As we know, the artificial composite materials may

be reinforced with disperse particles, fibres, layers (laminae) or three-dimensional framework. But in biological tissue, e.g. compact bone tissue, there exist all of these reinforcement forms. This gives a high resistibility of bone tissue to different loads and, especially, high resistance to crack propagation.

Table. Different parameters of the mechanical properties of 3-dimensionally glass fibre reinforced plastic (3D GFP) and compact bone tissue

Parameter	3D GFP	Compact bone tissue
E_1/G_{12}	3–10	3–5
$\sigma^{*+}_{11}/\sigma^{*}_{13}$	2–5	2
E_1/E_3	1–3	2–3
$\sigma^{*+}_{11}/\sigma^{*-}_{33}$	1	1

The parameters in the Table are the following: E_1 – the modulus of elasticity along the longitudinal axis of bone; E_3 – the modulus of elasticity along the radial axes of bone; G_{12} – the shear modulus in the plane formed by longitudinal and transversal axes of bone; σ^{*}_{11} – the ultimate tensile stress along the longitudinal axis of bone; σ^{*-}_{33} – the ultimate compression stress along the radial axis of bone; σ^{*}_{13} – the ultimate shear stress in the plane formed by longitudinal and radial axes of bone.

As it is seen from the Table the relative (specific) parameters of the mechanical properties of compact bone tissue are rather similar with those of 3D glass fibre reinforced plastics. Such spatially reinforced material has a very high resistance to crack propagation and cyclic loading.

The *principle of optimum* considers the existence of optimal combination of different mechanical properties and biochemical composition of biological tissue with respect to their functioning peculiarities. Nevertheless, it is worth mentioning that not everything in the structure of biological tissue is optimal with respect to the realisation of certain functions, e.g. the resistance to the mechanical loads. And the reason is simple: the biological tissue is fulfilling not only mechanical functions but also many different biological functions. Therefore, the mechanical and physical properties, and also the structure of biological tissue have been developed during the long-term evolution process optimally with respect to the fulfillment of different diverse functions.

3. Structural levels of the bone tissue

The determination of the mechanical properties of biological tissue *in vitro* is usually carried out on the special testing samples. The size and shape of these samples depend significantly upon the material structure.

In the continuum mechanics it is assumed that a solid material continuously occupies a geometrical volume of sample both before and after the deformation. Consequently, the strains and displacements of an individual point of sample are assumed to be continuous functions of co-ordinates. Therefore, in this case we disregard the real discrete structure of substance. The sample in the analysis of its stress–strain state is conditionally divided into micro-particles, which are tightly adjoined to each other and have the same properties as the whole sample.

Selection of the shape and size of samples for mechanical testing depends significantly upon the material structure.

In the continuum mechanics it is assumed that a solid material continuously occupies a geometrical volume of a body both, before and after the deformation. Consequently, the strains and displacements of an individual point of body are assumed to be the continuous functions of co-ordinates and, therefore, the real discrete structure of substance is disregard. Such a body in the analysis of its stress–strain state is conditionally divided in micro-particles, which are tightly adjoined to each other and have the same properties as the whole body.

The application of the model of solid mechanics to the analysis of uniform materials is related with the neglecting of the molecular structure of real body and the transition to the phenomenological description of its properties.

For composite materials, like many biological tissues, the transition to the continuum model of solid is more complicated. The mechanical properties of composite material depend upon both the properties of components and the character of their interaction. Consideration of the behaviour of each reinforcing fibre separately, in general, is not advisable because it may cause the significant mathematical difficulties during analysis. Therefore, it is assumed that these fibres are "redistributed" uniformly over the whole volume of a specimen. As a result, instead of a complex composite heterogeneous solid we obtain a uniform solid with the given new integral properties.

It is possible to apply such a method of "redistribution" if the size of structural level and the distance between neighbouring elements of this level are small compared with the characteristic size of macro-sample for mechanical testing and with the distances at which the functions determining the stress–strain state of structural elements may change significantly. These conditions are fulfilled if the diameter or thickness of specimen is by one–two orders higher than a characteristic size of structural level.

Existence of different structural levels we can distinguish practically in all biological tissues – in bone tissue, muscle, tendon, blood vessel, etc.

The structure of the reinforcement of compact bone tissue is much more complicated compared with the structure of traditional artificial composite materials. This phenomenon is related to the fact that there is a continuous renewal of the main biochemical compounds and remodelling of bone structure at the different structural levels. The analysis of the structure of bone tissue and the mechanical behaviour of

separate structural elements allow us to consider this material as a composite with five structural levels [1].

On the *first structural level* the characteristic components are macromolecules of tropocollagen and crystals of hydroxyapatite. The molecule of tropocollagen is formed by three left-spinned polypeptide chains. These chains are forming the right-spin helix stabilized by hydrogen bonds. Diameter of macromolecule is about 1.2–1.5 nm. As the *second structural level* we may consider micro-fibrils which are formed from five helically-wounded macromolecules of tropocollagen and the same mineral crystals. Diameter of micro-fibril is about 3.5 nm. These two levels represent the structural components of bone tissue on the molecular scale and, therefore, substitution of this heterogeneous solid for a uniform one could cause certain errors in the mathematical or numerical modelling.

The *third structural level* embraces relatively thick fibres with the diameters of about 100–200 nm consisting of many collagen micro-fibrils and mineral crystals tightly connected with them. These collagen-mineral fibres are embedded into physically non-linear inter-fibril substance (matrix) and, in fact, may be considered as the main reinforcing component of bone tissue.

The *fourth structural level* is formed by lamellae, the smallest self-dependent structural elements of bone tissue. They consist of many collagen-mineral fibres oriented in a certain direction depending on the particular lamellae. The thickness of separate lamella is about 4–7 μm. The *fifth level* is represented by osteons – the specific structural elements generated around the small blood vessels in the bone during its growth. The diameter of osteon is about 0.25 mm. These two last structural levels correspond to the actual structural components of bone.

Thus, viewing the compact bone tissue as a composite solid we have come to the conclusion that the third level is the basic, characteristic structural level. This conclusion allows us to determine the minimum thickness of test sample at which the tested material could be considered as the compact bone tissue. This thickness, as it was mentioned above, has to be, at least, 1–2 orders larger than the characteristic size of the basic structural level.

It means that in the experimental investigation of bone tissue with lamellar and osteonal structures, the minimum dimension of sample cross-section has to be no less than 1.5–2.0 mm. Only in the specific well-founded cases, the use of smaller specimens may be admitted.

4. Preparation of specimen

For investigation of the mechanical behaviour of human fresh bone tissue the bone has to be taken during autopsy no later than 1–2 days after one's death. The preparation of specimens consists in cutting a bone in beforehand-determined pieces and the processing of test samples on the milling machine or lathe. The processing of bone

tissue is accomplished at the low cutting rates and with a continuous cooling by glycerin emulsion to decrease the possibility of bone tissue overheating.

The bones themselves after autopsy and the prepared test samples of bone tissue until the experiment have to be kept in a manner preserving their moisture. The best way to fulfil this condition is keeping the specimens in closed polyethylene packages at the temperature from –4 to –7 °C. The period of storage from the moment of autopsy until the experiment is recommended to be not longer than two weeks. The samples before the experiment have to be kept in the same packages at the room temperature (+20 ^{0}C) for at least 3 hours to guarantee their uniform warming up. The temperature and moisture of environment during an experiment must be maintained constant.

If the experiment is carried out at the regular room temperature of 20±1 °C and moisture of 65±2% it is needed to protect the test sample from drying-out (especially in long-term loading) by wrapping it into a moist fabric or cotton wool. The best way to maintain the given temperature (37±0.5 °C) and moisture (80±2%) conditions is by using a special testing chamber. In such a case the bone tissue is under conditions that are very similar to its physiological conditions.

5. Effect of moisture

The change of bone moisture conditions may cause some significant alterations in the character of both the deformation and the fracture. Therefore, its disregarding may cause a large scattering of experimental data and even lead to a false opinion of the influence of moisture on the mechanical properties of bone tissue.

It is especially significant to keep the needed moisture conditions constant during testing of soft biological tissue because neglecting this factor could lead to completely inadequate results.

Specimens of compact bone tissue from the human (20–30 year old) left tibia were tested for tension at different relative moisture contents W and strain rates ε'_{11} [2]. It has been found that the form of stress–strain curves depends significantly upon the level of W. The completely dried bone tissue samples ($W = 0\%$) in a whole range of a low strain rate ($\varepsilon'_{11} = 0.0007$ sec^{-1}) has a linear stress–strain relationship up to the fracture. The increase of ε'_{11} leads to the manifesting of material's physical non-linearity, and the water-saturated bone tissue has the stress–strain curve, which is similar to the typical stress–strain curve of elastic-plastic material with a distinct yield point.

With the increase of the moisture content of bone tissue both the initial modulus of elasticity E_1 and the tangent modulus of elasticity at the moment of fracture decrease, but the ultimate strain ε^*_{11} increases. There is also some decrease of the ultimate stress σ^*_{11}: at $W = 10.5\%$ the value of σ^*_{11} is 30% lower than at $W = 2.5\%$.

At the same time, some structural alterations take place in the material with the increase of its moisture. These alterations increase both linear dimensions and volume. The water that penetrates into bone tissue causes the hydrostatic tension in bone matrix. Because of the orientation of reinforcing collagen fibres mainly along the longitudinal axis of bone, the swelling in this direction is about 4–5 times less than in the transversal and radial directions.

The mode of bone fracture is also changing with the alterations of moisture [3–4]. When the bone tissue is subjected to drying-out its collagen matrix becomes brittle and, because of the presence of shrinkage stresses, the micro-cracks appear. This leads to the brittle mode of tensile fracture. The angle between the fracture plane and the longitudinal axis of test sample is about 90°.

The increase of moisture content causes the transfer of fracture mode from the brittle to the ductile one: the bone tissue behaves like a visco-elastic material. In a moist bone the hydroxyapatite crystals deform elastically, while the behaviour of collagen matrix is visco-elastic. The fracture surface is inclined at the angle of 45° to the axis of specimen, and the pulling out of separate osteons or its parts from the bone matrix takes place, i.e. the ductile fracture appears because of the presence of shear stresses.

6. Variation of elastic parameters

The mechanical properties of bone tissue of different bones for one and the same individual may differ depending upon its functions in the body. Thus, the ultimate compression stress of the wet compact bone tissue of human tibia is 167 MPa, but of human radius –115 MPa. At the same time the ultimate tensile stresses of these bones are 122 and 149 MPa, respectively.

The experimental data indicate that the statistically significant differences exist even between the parameters of the mechanical properties of bone tissue obtained from the bones of the left-hand or the right-hand side of human body.

In this paper we shall restrict our analysis to the heterogeneity of the bone tissue of human tibia over the zones of its cross-section determined by the complex test method. Complex method means that on one and the same specimen the non-destructive and destructive tests, the analysis of structural parameters and the analysis of biochemical composition were carried out.

The cross-section of human left-hand tibia is divided into six zones: three corner zones (1, 3, 5) and three inter-corner zones (2, 4, 6). The testing methods for determination of different parameters of the mechanical properties of bone tissue over these zones are given in detail in [1] and in its references. Here we shall discuss the obtained results only.

The moduli of elasticity were determined, at first, by means of non-destructive testing. Specimens were cut out along the longitudinal axis of bone. The modulus of elasticity ($E_{1(\text{n.f.})}$) was determined from the measurement of the specimen's natural

flexural frequency of vibration, and the modulus ($E_{1(150)}$) – from the measurement of the velocity of ultrasound during scanning of specimen by longitudinal waves at the frequency of 150 kHz. The distribution of these values over the zones of tibia cross-section is rather uniform (coefficient of correlation $r = 0.96$ at the level of the significance $p < 0.0005$). The minimum values of both moduli are in the posterior zone 4 and the maximum values – in the lateral corner zone 5, but in each zone the value of $E_{1(150)}$ is larger than $E_{1(n.f.)}$. The heterogeneity of anisotropy of the moduli of elasticity $E_{i(1670)}$ and $E_{i(5000)}$ were determined by ultrasonic longitudinal wave scanning of cubic bone samples at the frequencies of 1670 and 5000 kHz, but the shear moduli $G_{ij(450)}$ ($i, j = 1, 2, 3$) – by ultrasonic shear wave scanning of the same samples at a frequency of 450 kHz. The average values of these moduli over the zones of tibia cross-section are the following (in GPa): $E_{1(n.f.)} = 18.50 \pm 0.97$; $E_{1(150)} = 19.80 \pm 1.14$; $E_{1(1670)} = 31.32 \pm 1.75$; $E_{2(1670)} = 20.01 \pm 1.44$; $E_{3(1670)} = 18.22 \pm 1.72$; $E_{1(5000)} = 39.20 \pm 3.65$; $G_{12(n.f.)} = 4.75 \pm 0.37$; $G_{12(450)} = 5.20 \pm 0.31$; $G_{23(450)} = 3.36 \pm 0.22$; $G_{31(450)} = 4.69 \pm 0.29$.

The given data are related to the human bone tissue at the age of 25–44 years.

The variations of $E_{2(1670)}$ and $E_{3(1670)}$ over the zones of cross-section are more non-uniform than $E_{1(1670)}$. Variations of $G_{ij(450)}$ are even more expressed: for example, $G_{23(450)}$ has statistically significant differences between each adjacent zone with exception of 6–1.

The experimental determination of the parameters of mechanical properties of compact bone tissue as orthotropic material by destructive tensile test method is more complicated. The moduli of elasticity E_i along three axes of material symmetry ($i = 1, 2, 3$) and Poisson's ratios μ_{ij} ($i, j = 1, 2, 3$; $i \neq j$) of the compact bone tissue of human tibia at the age of 25–34 years were determined [1]. The average values of the moduli of elasticity E_i over the cross-section of tibia are the following (in GPa): $E_1 = 18.7 \pm 0.4$, $E_2 = 8.7 \pm 0.3$, $E_3 = 7.0 \pm 0.2$.

Distribution of E_1 over the zones of tibia cross-section is non-uniform [5]: the statistically significant differences are found between each adjacent zone with exception of 6–1. In zone 6 modulus E_1 has the maximum value (20.6 ± 0.4 GPa) but in zone 4 it has the minimum (16.3 ± 0.3 GPa). The character of the distribution of E_1 is about the same as that of $E_{1(150)}$ ($r = 0.75$ at $p < 0.025$). The non-uniformity of E_2 and E_3 is also more expressed than non-uniformity of $E_{2(1670)}$ and $E_{3(1670)}$.

This result gives us a possibility to make a conclusion that the destructive tension test reveals the heterogeneity of tibia more distinctively than the non-destructive testing methods. The degree of anisotropy $E_1 : E_2 : E_3$ is changing non-uniformly over the zones of cross-section. The more distinctive anisotropy is found in zone 6, where $E_1 : E_2 : E_3 = 1 : 0.4 : 0.3$ (i.e., $E_2/E_1 = 0.4$; $E_3/E_1 = 0.3$).

The values of Poisson's ratios over the zones of tibia cross-section are the following: $\mu_{12} = 0.31 \pm 0.01$, $\mu_{13} = 0.31 \pm 0.01$, $\mu_{21} = 0.14 \pm 0.01$, $\mu_{31} = 0.12 \pm 0.01$, $\mu_{23} = 0.62 \pm 0.02$, $\mu_{32} = 0.49 \pm 0.02$. The character of the changes of Poisson's ratios μ_{12} and μ_{13} over the zones of cross-section is uniform (significant difference is found for μ_{12} between adjacent zones 5 and 6 only). However, Poisson's ratios μ_{31} and μ_{32}, which

characterize the transverse strains in tension along the radial axis x_3, are distributed non-uniformly. In general, the non-uniformity of μ_{ij} is more distinctive between adjacent zones 5 and 6. In zone 5 the largest ($\mu_{23} = 0.767 \pm 0.022$), but in zone 6 the smallest ($\mu_{31} = 0.092 \pm 0.004$) values of μ_{ij} over the zones of tibia cross-section are also found.

The determination of the shear moduli G_{ij} were carried out in the torsional tests of cylindrical specimens of compact bone tissue. The average values of the shear moduli over the cross-section of man left tibia in the age group 45–59 years are the following (in GPa): $G_{12} = 5.01 \pm 0.17$, $G_{23} = 2.46 \pm 0.14$, $G_{31} = 3.63 \pm 0.18$. The variation of shear moduli G_{ij} is more expressed between zones 4 and 5: each of three moduli at this age group has a statistically significant difference.

7. Variation of strength parameters

The average values of the ultimate tensile stress and strain over the cross-section of tibia (age group – 25–44 years) are the following (in kPa): $\sigma_{11}^* = 130.9 \pm 4.1$, $\sigma_{22}^* = 17.3 \pm 0.6$, $\sigma_{33}^* = 13.2 \pm 0.5$; $\varepsilon_{11}^* = 0.86 \pm 0.03\%$, $\varepsilon_{22}^* = 0.23 \pm 0.03\%$, $\varepsilon_{33}^* = 0.21 \pm 0.01\%$.

The variations of ultimate tensile stresses over the zones of tibia cross-section are more expressed for σ_{33}^* – a significant difference does not exist between zones 6–1, 1–2 only (i.e., in the anterior corner of bone). The less expressed heterogeneity is found for σ_{11}^*. It has a statistically significant difference between zones 3–4 and 4–5 and it is in good correlation with the distribution of E_1 over the zones of cross-section ($r = 0.96$).

Variation of ultimate tensile strains is also non-uniform. The largest heterogeneity is found between zones 4–5, 5–6, the smallest between zones 6, 1 and 2.

The moduli of elasticity change their values during the loading process, depending upon the stress. The character of heterogeneity of the secant modulus of elasticity E_1^{sec} over the zones of tibia cross-section is constant during the loading. However, the modulus E_2^{sec} has very marked changes in its distribution over zones with the increase of loads.

8. Variation of biochemical composition

The heterogeneity of the mechanical properties of bone tissue over the zones of tibia cross-section is caused by both the differences in structure and the heterogeneity of the biochemical composition. In order to evaluate these changes of biochemical composition, a special analysis was performed [1] on the same samples which were tested mechanically.

The determination of the quantitative composition of each bone tissue compounds by a biochemical analysis is difficult. However, there is the possibility to judge some

relative changes of them over the zones of cross-section on the basis of data obtained by analysis of certain substances, which are easier to determine. Thus, on the basis of oxyprolene content Q_{ox} we have evaluated the content of fibrillar protein – collagen Q_{col} ($Q_{col} = 7.5Q_{ox}$). Similarly, the changes of the hydroxyapatite content over the zones of bone cross-section were determined from the changes of phosphorus Q_P, the concentration of non-collagen proteins – from tyrozine, the concentration of matrix – from hexozamine Q_{hex}.

It was found that the anterior corner zone 1 had a minimum of phosphorus and a high concentration of hexozamine. In the posterior zone 4 the hexozamine content had a minimum, but the oxyprolene – a maximum. In the corner zones 3 and 5 the biochemical substances had a uniform distribution – there were no maximum or minimum values of them over the zones of cross-section.

The high positive correlation (the coefficient of Spearman's rank correlation $r_S = 0.75$) exists between the concentrations of oxyprolene and phosphorus. This confirms our assumption that the collagen-mineral fibres, where exists the close connection between collagen microfibrils and hydroxyapatite, have to be considered as the main structural level in bone tissue. Besides, Q_{ox} and Q_P are strongly correlated with the density of bone tissue ($r_S = 0.86$ and 0.64, respectively), i.e., with one of the parameters of bone structure.

The modulus of elasticity E_1 and the ultimate tensile stress σ_{11}^* have a high negative correlation with Q_{ox} ($r_S = -0.86$ and -0.82) and a less expressed negative correlation with Q_P ($r_S = -0.5$ and -0.54). The ultimate tensile strains ε_{11}^*, ε_{22}^* and ε_{33}^* have a high positive correlation with Q_{hex} ($r_S = 0.82$; 0.86; 0.64, respectively). Q_{hex} has also a high positive correlation with $G_{12(450)}$ ($r_S = 0.89$).

9. Change of heterogeneity with age

Both the biochemical composition and the structure of bone tissue change with age. This causes certain changes in its mechanical properties, too.

With the process of ageing the modulus of elasticity E_1 (on average over the zones of tibia cross-section) increases up to the age of 18–22 and then starts to decrease gradually. The shear moduli G_{ij} and strength parameters change similarly. At the same time there is also some change of their distribution over the zones of cross-section. Especially interesting is a change of the location of maximum and minimum values of G_{12} over the zones with the process of ageing. It turns out that for left-hand human tibia there is a clock-wise "turning" of zones, where the statistically significant difference between the values of G_{12} exists. As a result, the maximum of G_{12} shifts from one corner zone to the other corner zone, but the minimum values of G_{12} shift from one inter-corner zone to the other inter-corner zone. The knowledge of this phenomenon is significant for the correct calculation of the stress and strain states in bone at different ages.

10. Effect of strain rate

The influence of strain rate (from 10^{-5} to 1 sec^{-1}) on the mechanical behaviour of compact bone tissue under tension under different moisture conditions was analysed [2, 6]. It was found that at the constant moisture W the change of ε'_{11} in this given range did not affect the character of stress–strain curves, but led to the increase of ultimate stress σ^*_{11}.

If for the bone tissue with the moisture content $W = 2.5\%$ the stress–strain curves σ_{11}–ε_{11} at different strain rates became distinctive only in the region, which was close to ultimate stress, this distinction at larger moisture contents was seen already at lower stress levels. The average values of the modulus of elasticity E_1 at the moisture $W = 2.5$ and 8.5% were 21.8 and 18.8 GPa, respectively.

For the water-saturated bone tissue ($W = 10.5\%$) the stress–strain curve σ_{11}–ε_{11} might be represented by two quasilinear regions, and the modulus of elasticity E_1 in the range of ε'_{11} from 10^{-3} to 10^{-1} was practically independent of σ_{11}. Its average value is 18 GPa. The tangent modulus of elasticity in the second region of deformation was independent of σ_{11} in the investigated range of its change.

The change of σ^*_{11} vs. $\log \varepsilon'_{11}$ was linear, but it depended upon the moisture content W.

The analysis of the tensile fracture surface of water-saturated bone tissue ($W = 10.5\%$) showed that in the investigated range of ε'_{11} only the ductile character of fracture existed. During such a fracture the pulling out of some structural elements from the matrix is taking place – at low strain rates the large structural units are pulled out, but at the high strain rates – only the small ones. In the compression and bending tests some transition from the ductile fracture mode to a brittle one takes place.

11. Creep

To determine the effect of long-term loading on the behaviour of compact bone tissue the creep test was carried out [6]. The samples of compact bone tissue were subjected to tension in the special camera with fixed moisture content (90%) at six constant stress levels: $\sigma_{11}/\sigma^*_{11} = 0.2$; 0.3; 0.4; 0.5; 0.6 and 0.7.

At the stress levels $\sigma_{11}/\sigma^*_{11} = 0.6$ and 0.7 the value of creep strain ε^{cr}_{11} increased intensively and in some cases samples fractured during this test.

A significant residual strain after the passive creep process appeared when $\sigma_{11} > 0.4\sigma^*_{11}$. Up to this stress level the behaviour of bone tissue may be considered as that of a linear visco-elastic solid, and after this stress level – as a non-linear visco-elastic solid.

The creep properties depend significantly upon the conditions of the preservation of specimen and testing. The specimens that were preserved at the room temperature (20 °C) and moisture of 65% had the smallest compliance (on average over the zones of cross-section $\varepsilon^{cr}_{11} = 0.12\varepsilon^{e}_{11}$ at each stress level after the creep during 200 min). The

samples which were kept for 30 days in polyethylene packages at a temperature range from –4 °C to –7 °C or in the physiological solution and tested under the wet conditions of environment (W = 90%) had $\varepsilon_{11}^{cr} = 0.23\,\varepsilon_{11}^{e}$ at the stress level $\sigma_{11}/\sigma_{11}^{*}$ = 0.7. The creep strain increased sharply in samples that were kept in the physiological solution and during testing were under moist conditions ($\varepsilon_{11}^{cr} = 1.5\,\varepsilon_{11}^{e}$ at the stress level $\sigma_{11}/\sigma_{11}^{*} = 0.5$).

The creep properties of bone tissue depend upon the age of man: in the young bone tissue the visco-elastic behaviour is more expressed than in the old one. After the age of 50 the creep strain practically remains constant. The distribution of creep strain ε_{11}^{cr} over the zones of cross-section for young bone tissue is non-uniform. The process of ageing leads to the "smoothing" of this non-uniformity and, therefore, to the poorer ability of the bone to be rheologically adapted to the mechanical loads.

The increased creep of young bone tissue may be explained by a low degree of mineralization and, consequently, by a relatively large content of collagen. It should be mentioned that because of a large active surface of the mineral component and a rather large amount of water in young bone, an intensive exchange of ions between the mineral phase and the tissue fluid is possible. Therefore, the young bones due to a high activity of different processes in them have an ability to react fast even to small mechanical loads. The increase of crystal size and its distinctive orientation with age cause the decrease of the active surface of mineral component and, therefore, may influence the diffusion rate and the intensity of ion exchange. The bone becomes chemically less active and the processes of resorption prevail over the processes of bone formation. This could be one of the reasons why the heterogeneity of creep properties over the zones of cross-section decreases with age.

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