# BIAXIAL TENSION TESTS WITH SOFT TISSUES OF ARTERIAL WALL

Miroslav Zemánek, Jiří Burša, Michal Děták*

*The paper presents results of mechanical testing of soft tissues (arterial walls) under biaxial stress conditions and analysis of the influence of some factors, such as specimen location, preconditioning, etc. Soft tissues are pseudoelastic materials, modelled mostly as hyperelastic, either isotropic or anisotropic ones. Therefore multiaxial (biaxial) mechanical tests are required for a credible identification of their mechanical parameters. As living tissue proporties change with time after excision and exhibit also viscoelastic behaviour, a much more specialized equipment is needed to perform biaxial tests of soft tissues. A test rig for biaxial tests is presented in the paper and a pronounced influence of stress state character on the specimen behaviour during several first cycles is analyzed.*

Keywords: *soft tissue, aortic wall, hyperelastic constitutive models, biaxial tension tests*

## 1. Introduction

Contrary to linear elastic materials, uniaxial tension tests are not sufficient for a reliable determination of parameters of hyperelastic constitutive models aiming to predict material bahaviour under different types of biaxial stress-strain states [4]. Various types of biaxial mechanical tests are required to quantify mechanical properties of hyperelastic materials, no matter how they are modelled (isotropic or anisotropic). In cooperation of our institute with Camea s.r.o. company, an experimental rig for biaxial testing of hyperelastic materials (elastomers and soft tissues) was designed and produced. This testing rig enables us biaxial tension tests with independent control of displacement rates in both mutually perpendicular directions of the specimen. This paper presents the procedure of biaxial tension tests with soft tissues (aortic walls) and assessment of significance of some influencing factors, in particular temperature, specimen conservation, preconditioning of the specimens, loading rate and specimen location 'in situ' (in the body).

### 1.1. Motivation

Knowledge on biomechanical properties of arterial walls is necessary for credible description of their constitutive behaviour in Finite Element Analyses (FEA). Computational modelling enables us a better prediction of the outcome of interventional treatments (e.g. balloon angioplasty). The paper summarizes our work carried out in mechanical testing of aortic tissues and analyzes our experimental results in comparison with data available in literature.

* Ing. M. Zemánek, doc. Ing. J. Burša, Ph.D., Ing. M. Děták, Institute of Solid Mechanics, Mechatronics and Biomechanics, Faculty of Mechanical Engineering, 616 69 Brno

## 2. Biaxial testing rig

The testing rig (Fig. 1) consists of a bedplate carrying two orthogonal ball screws, equiped with force gauges, two servo motors and four carriages ensuring symmetric biaxial deformation of the specimen and a programmable CCD camera located on a support stand. The specimen can be immersed in physiological saline solution with controlled temperature; the whole test is driven by a PC using a tailored software system. For clamping of specimens, four clips are attached to each of the carriages by a system of levers. The CCD camera is used for contactless evaluation of deformations. The independent control of displacements in both loading directions enables us to obtain stress-strain characteristic for various types of biaxial stress states. It is possible to obtain stress-strain characteristics in the following types of tests:

a) **equibiaxial tension tests** – equal strains in both loading directions
b) **planar tension tests** – uniaxial extension in either '1' or '2' direction with constrained (zero) transversal contraction
c) **proportional tension tests** – biaxial loading with mutually proportional strain components in both loading directions
d) **tension tests with constant transversal strain** – increasing load in either '1' or '2' direction and a constant (non-zero) strain in the other one
e) **uniaxial tension test** – loading only in either '1' or '2' direction with free transversal contraction

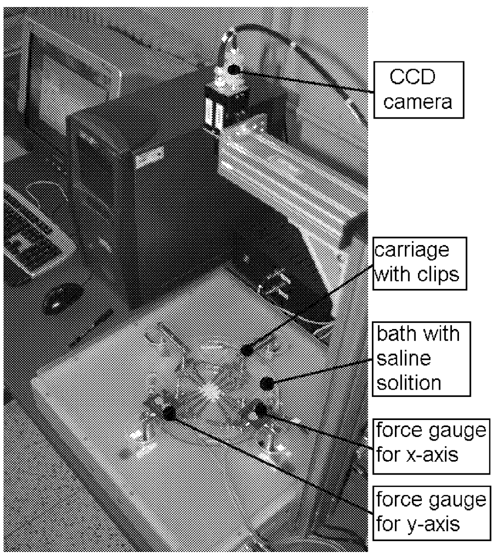


*Fig.1: General view of the testing rig*

## 3. Specimen preparation

Mechanical tests of soft tissues are realized 'in vitro' using square or rectangular specimens. The specimens were cut-out from porcine thoracic aortas between aortic arc and renal arteries. Porcine thoracic aorta was chosen for its simple availability and similarity to human aortic tissue. Every edge of the specimen must be clamped by two or four clips to achieve a uniform distribution of load along the specimen width. Plastic templates are used for keeping the defined spacing distance between opposite and neighbouring clips (see Fig. 2). The clips must ensure holding of the specimen without its damage, therefore the torque used for tightening of the clip screws should be controlled. The tissue thickness was measured manually in three different locations and an average value was calculated and used for evalutation. The reference markers (four black points) were drawn on the specimen surface with alcohol-based permanent ink and then the specimen was immersed into the physiological saline solution (0.9 % NaCl) with controlled temperature (Fig. 3). Preload of the specimens was realized by a maximum total load of 0.5 N; after preloading the specimen was loaded by a constant strain rate. During the test, positions of reference points were recorded by the CCD camera (together with the measured force values) and the data were used for further processing by a special authorized software, making an off-line image analysis. The influence of several factors was tested in the experiments presented in the following chapter.

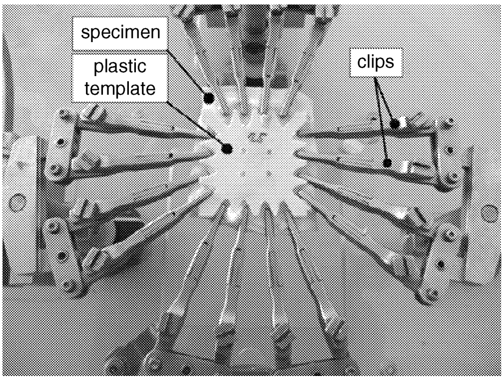


*Fig.2: Clamping of specimen using a plastic template*

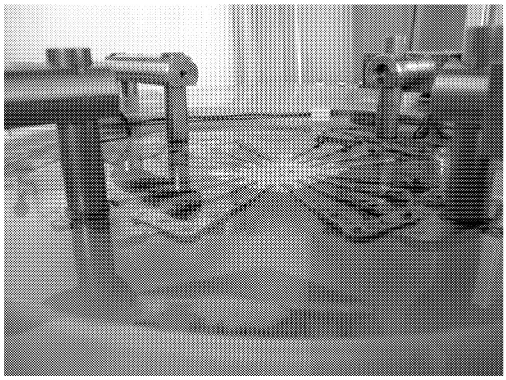

*Fig.3: Specimen immersed in physiological saline solution*

## 4. Results of testing under influence of various factors

All the resulting material characteristics are presented in the coordinates true stress vs. stretch ratio; only in fig. 4 natural strains were calculated from the stretches.

### 4.1. Influence of temperature changes

Specimens were tested at temperatures 30 °C and 37 °C after having balanced their temperature for several minutes in the physiological saline solution. Temperature increase by 1 °C results in a stiffness decrease of ∼5 % (Fig. 4); this corresponds to values from literature [9]. The standard temperature of 37 °C is generally used in mechanical testing of arteries [1], [10] as well as in all the following tests.

### 4.2. Influence of specimen conservation

Arterial specimens are commonly preserved using refrigeration and freezing. Rare studies examined the effect of freezing and some of them (for example [5]–[8]) presented ambiguous results. To test the influence of freezing process, fresh specimens of arterial wall were

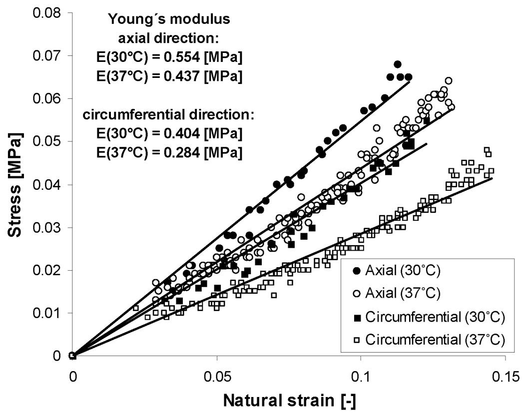


*Fig.4: Equibiaxial stress-natural strain responses for different temperatures*

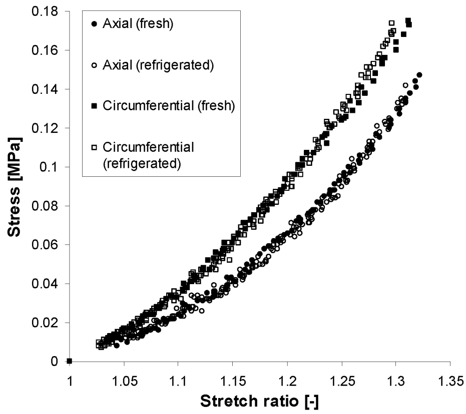


*Fig.5: Equibiaxial stress-stretch responses for fresh and refrigerated specimen*

tested within 2 hours from preparation (ectomy) and then refrigerated at $-20\,°\mathrm{C}$ and tested overnight again under the same conditions. We concluded that specimens after refrigeration and thawing have shown no significant changes in mechanical properties compared with the fresh ones (Fig. 5). The maximum relative stress differences are 5 and 7.5 % for axial and circumferential directions, respectively. The influence of longer refrigeration of specimens (in weeks or months) was not tested.

### 4.3. Influence of loading rate

Generally the tension response of soft tissues depends on the strain rate. Most soft tissues, among others arterial wall as well, appear to behave nearly incompressibly and in a markedly viscoelastic manner [9]. The physiological strain rate of healthy arterial wall is about $1\,\mathrm{s}^{-1}$ (in the systolic phase of cardiac cycle). This value depends on the artery location, age, artery and heart disease etc. Our rig enables us testing at strain rates from $0.004\,\mathrm{s}^{-1}$ up to $0.100\,\mathrm{s}^{-1}$ only. The stress-stretch responses in this strain rate range are shown in Fig. 6. The results are analyzed in section 5.3.

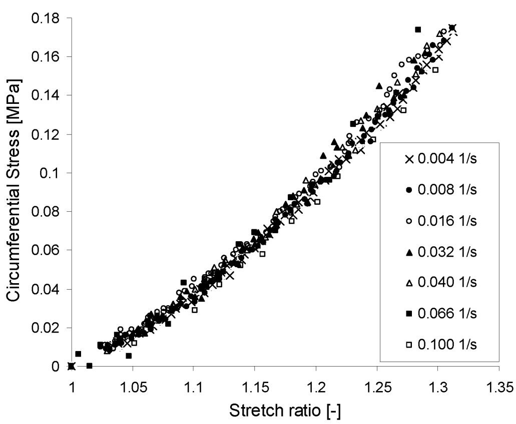


*Fig.6: Equibiaxial stress-stretch responses for different loading rates*

### 4.4. Influence of preconditioning

Cyclic stress response during tension testing has been described in a number of biomechanical books and journals (e.g. [2]–[4], [9] and [10]). The specimens had been subjected to loading and unloading cycles until the stress softening effect diminished and the material exhibited a nearly repeatable cyclic behaviour. The material is then said to be 'preconditioned'. An arterial wall consists of three major layers: the innermost intima, the media and the outermost adventitia. The adventitia consists of fibrous components (collagen and elastin fibres) and a non-fibrous matrix. The results published in [3] show a direct relation between changes in orientation and extension of the collagen fibres under load. Number of the necessary preconditioning cycles during uniaxial tensile tests depends on the origin of the specimen (species, localization, age, type of artery, etc.). In our experiments, the influence of preconditioning of specimens was tested in equibiaxial (Fig. 7), planar (Fig. 8) and uniaxial (Fig. 9) tension tests. The results are analyzed in section 5.1.

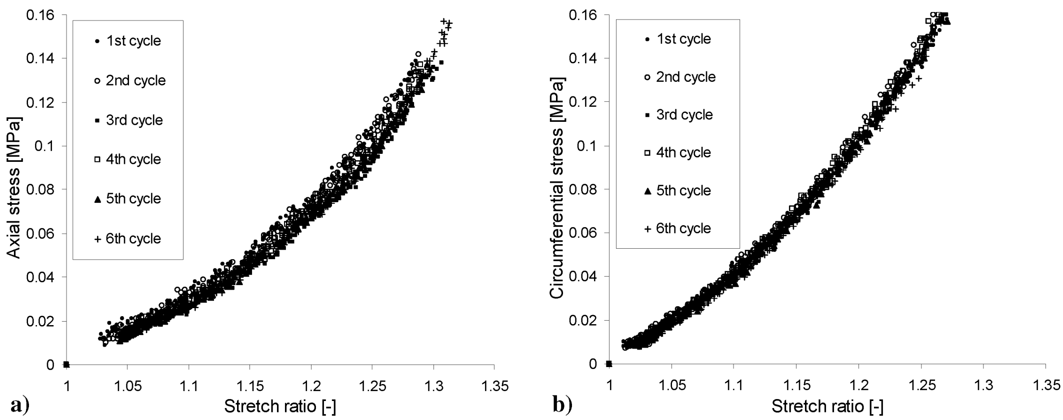


*Fig.7: Cyclic stress-stretch responses in equibiaxial tension tests a) axial direction; b) circumferential direction*

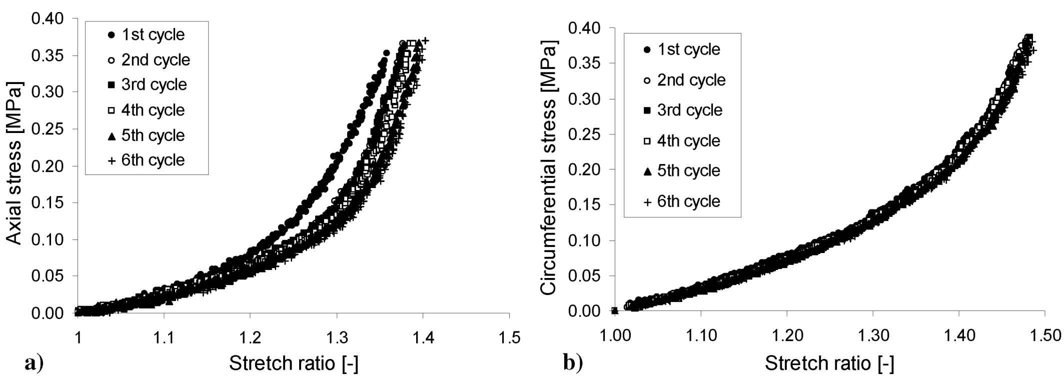


*Fig.8: Cyclic stress-stretch responses in planar tension tests a) axial direction; b) circumferential direction*

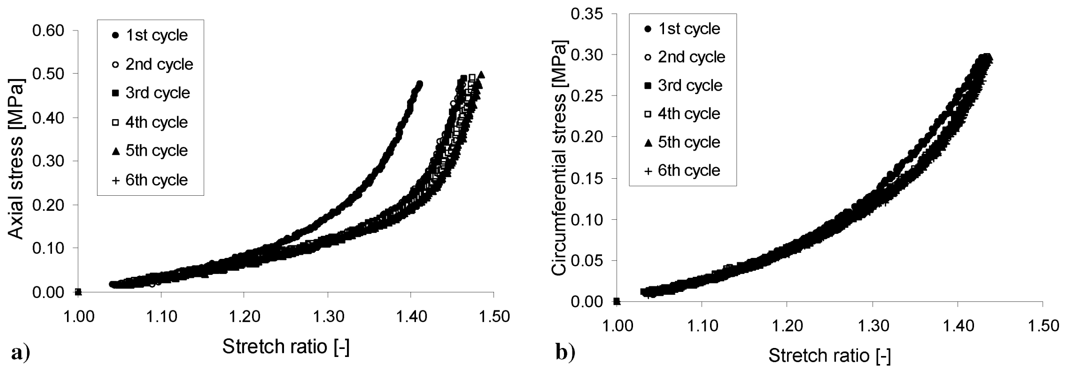


*Fig.9: Cyclic stress-stretch responses during uniaxial tension test a) axial direction; b) circumferential direction*

### 4.5. Influence of specimen location

The influence of specimen location was also tested experimentally. It was possible to obtain only three or four specimens (approximatelly 40×40 mm) from one thoracic aorta and only one at each axial location; the difference in axial location of neighbouring specimens was about 50 mm. The stress-stretch responses of four specimens in various axial locations are shown in Fig. 10. The results are analyzed in the following section, par. 5.2.

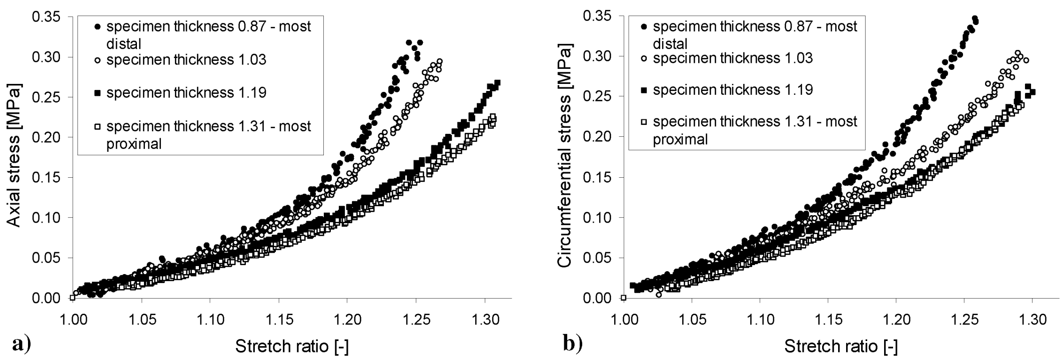


*Fig.10: Equibiaxial stress-stretch responses in various locations along the thoracic aorta; a) axial direction; b) circumferential direction*

## 5. Analysis of results

### 5.1. Influence of preconditioning

The results of the preconditioning tests are summarized in Tab. 1. We can conclude that the effect of preconditioning is negligible up to stretch ratio $\lambda = 1.35$ for equibiaxial tension test. (Higher stretch values have not been tested because of the risk of rupture in the vicinity of clamps – the highest stress concentration occurs in the corner of the specimen between the neighbouring clamps moving in perpendicular directions.) No significant changes in stress-strain relations occurred during the first two cycles (Fig. 7). The maximal relative stress differences between the 1$^{st}$ and 2$^{nd}$ cycles are 4.8 % and 2.9 % for axial and circumferential directions, respectively. The maximal relative stress differences between the

1st and 6th cycles are 9.2 % and 6.2 % for axial and circumferential directions, respectively. On the contrary, the data obtained in the planar and uniaxial tension tests (Fig. 8, Fig.,9) show a more pronounced shift of loading curves among the first several cycles. Significant differences were found between the 1st and 2nd cycles. In axial direction, the maximal relative stress differences are 24.0 % and 69.5 % in planar and uniaxial tension tests, respectively. Contrary to the first two cycles, the maximal relative stress differences between 5th and 6th cycles are 4.7 % and 3.5 % in planar and uniaxial tension tests, respectively. As these differences lie below the expected dispersion of results, six cycles were chosen as sufficient for preconditioning.

It can be concluded that changes in material behaviour during preconditioning are related to a pronounced alignment of the collagen fibres towards the applied force [3]. Therefore it appears realistic that no changes connected with preconditioning occur in equibiaxial tension, where no preferential direction of applied force exists.

| | stress differences [%] in the physiological range of loading | | | | | |
|---|---|---|---|---|---|---|
| | equibiaxial tension test | | planar tension test | | uniaxial tension test | |
| | axial direction | circ. direction | axial direction | circ. direction | axial direction | circ. direction |
| cycle 1–6 | 9.0–9.2 | 5.1–6.2 | 25.6–63.2 | 7.0–6.8 | 17.4–80.3 | 5.0–17.0 |
| cycle 1–2 | 3.7–4.8 | 3.0–2.9 | 16.7–24.0 | 5.6–2.4 | 15.7–69.5 | 5.0–13.3 |
| cycle 5–6 | 3.8–1.7 | 2.0–2.8 | 5.1–4.7 | 4.2–0.6 | 2.9–3.5 | 0.0–3.3 |

*Tab.1: Summary of stress differences between individual preconditioning cycles*

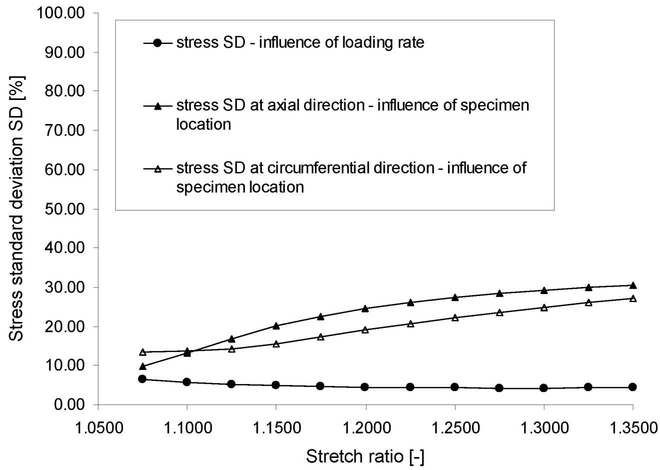


*Fig.11: Standard deviations [%] in stresses as function of stretch ratio [–]*

## 5.2. Influence of specimen location

It is evident from Fig. 10 that, in addition to the thickness, the stiffness has also changed significantly between the specimens of the same aorta; therefore the evaluation of material parameters is valid only for the particular axial location. The stress standard deviations over all measurements are 10 % up to 30 % of the average stress values (Fig. 11). However, it is necessary to carry out three types of tests of each specimen (equibiaxial and planar tension tests in either ‘1’ or ‘2’ principal directions) for identification of material parameters of constitutive equations describing biaxial behaviour of specimen. Therefore it is not possible to test the specimen over the full physiological range of loading because tearing could begin. Impossibility of getting more specimens from the same location represents a limitation in evaluation of biaxial tension tests and in identification of material parameters of constitutive models.

### 5.3. Influence of loading rate

Fig. 6 shows that the influence of loading rate is negligible for a range of 1–2 orders. The specimens have been tested under strain rates between $0.004\,\mathrm{s}^{-1}$ up to $0.100\,\mathrm{s}^{-1}$ and analyzed from a statistical point of view. Calculated stress standard deviations are in the range from 4.3 % up to 6.4 % of the average stress values (Fig. 11).

## 6. Conclusion

This study has attempted to systematically investigate the influence of some factors, such as boundary and loading conditions, on the results of mechanical tests of soft tissues. The conclusions are as follows:

- Influence of preconditioning is important in uniaxial and planar tension tests; on the contrary, in equibiaxial tension tests no preconditioning is necessary. The material behaviour during uniaxial tension tests is in accordance with the findings in literature [3]. It can be concluded that the changes in specimen stiffness are given by re-orientation of loadbearing fibres towards the direction of the first principal stresses; in equibiaxial tests the in-plane principal stresses are approximately equal, there is no reason for fibre reorientation and no significant preconditioning effect occurs.
- The influence of specimen location is important because the material properties change significantly along the thoracic aorta. Impossibility of getting more specimens from the same location represents a limitation in statistical evaluation of biaxial tension tests and in identification of material parameters of constitutive models.
- Influence of strain rate is negligible in the tested range ($0.004\,\mathrm{s}^{-1} \div 0.100\,\mathrm{s}^{-1}$).
- Influence of tissue freezing is negligible. The mechanical properties show no difference between fresh specimens and those after refrigeration.

The presented equipment and methodology enable us a credible testing of soft tissues under biaxial stress state conditions; these tests contribute to a more reliable identification of parameters of constitutive models used in FE models of tissues and organs of human body.

## References

[1] Schulze-Bauer C.A.J., Mörth C., Holzapfel. G.A.: Passive Biaxial Mechanical Response of Aged Human Iliac Arteries, J. Biomechanical Engineering 125 (2003) 395–406

[2] Holzapfel G.A., Sommer G., Gasser T.C., Regitnig P.: Determination of layer-specific mechanical properties of human coronary arteries with non-atherosclerotic intimal thickening, and related constitutive modeling, American J. Physiology – Heart Circulation Physiology 289 (2005) H2048–2058

[3] Schmid F., Sommer G., Rappolt M., Schulze-Bauer C.A.J., Regitnig P., Holzapfel G.A., Laggner P., Amenitsch H.: In situ tensile testing of human aortas by time-resolved small angle X-ray scattering, J. Synchrotron Radiation (2005) 727–733

[4] Lally C., Reid A.J., Prendergast P.J.: Elastic Behavior of Porcine Coronary Artery Tissue under Uniaxial and Equibiaxial Tension, Annals of Biomedical Engineering 32 (2004) 1355–1364
[5] Armentano R.L. et al.: An in vitro strudy of cryopreserved and fresh human arteries: a comparison with ePTFE prostheses and human arteries studied noninvasively in vivo, Cryobiology 52 (2006) 17–26
[6] Wang P. et al.: The viability, structure and mechanical properties of cryopreserved rabbit carotid artery, Cell preservation technology 3 (2005) 85–95
[7] Venkatasubramanian R.T. et al.: Effects of freezing and cryopreservation on the mechanical properties of arteries, Annals of Biomedical engineering 34 (2006) 823–832
[8] Stemper B.D. et al.: Mechanics of fresh, refrigerated and frozen arterial tissue, J. Surgical Research 139 (2007) 236–242
[9] Fung Y.C.: Biomechanics, Material Properties of Living Tissues, 2nd edition, Springer, 1993, New York Berlin Heidelberg
[10] Humphrey J.D.: Cardiovascular Solid Mechanics, Cells, Tissues, and Organs, Springer, 2002, New York Berlin Heidelberg

*Received in editor's office*: April 15, 2008
*Approved for publishing*: December 12, 2008