Mechanobiology of soft tissues: FT-Raman spectroscopic studies

Marlena Gąsior-Głogowska[1#], Małgorzata Komorowska[1], Jerzy Hanuza[2], Mirosław Mączka[3], Romuald Będziński[4#], Magdalena Kobielarz[4#]

[1] Institute of Biomedical Engineering and Instrumentation, Faculty of Fundamental Problems of Technology, Wrocław University of Technology, Wrocław, Poland; e-mail: marlena.gasior-glogowska@pwr.wroc.pl.

[2] Department of Bioorganic Chemistry, Wrocław University of Economics, Wrocław, Poland.

[3] Institute of Low Temperature and Structure Research, Polish Academy of Sciences, 50-950 Wrocław 2, Poland.

[4] Division of Biomedical Engineering and Experimental Mechanics, Institute of Machine Design and Operation, Faculty of Mechanical Engineering, Wrocław University of Technology, Wrocław, Poland.

[#] Regional Specialist Hospital in Wroclaw, Research and Development Center, ul. Kamieńskiego 73a, 51-124 Wrocław, Poland.

FT-Raman spectroscopy was used to investigate microstructural changes in the secondary protein structure of soft tissues subjected to increasing levels of macroscopic strain. Main protein bands at 938 cm^{-1} assigned as $_{\nu}(C_{\alpha}–C)$, 1668 cm^{-1} — amide I and 1268 cm^{-1} — amide III are sensitive to applied strain and undergo wavenumber shifting. Other main vibrational modes at 1004 cm^{-1} assigned to the phenyl ring breathing mode and 2940 cm^{-1} ($_{n}(CH_3,CH_2)$) remain unaltered.

Spectroscopic results were compared with the mechanical relations obtained from the standard protocol of uniaxial tensile tests carried out in a testing machine. A clear correlation between Raman band shifting and the level of mechanical stress was established. Initially the load is transferred through elastin and then gradually also by collagen. It was proved that transferring loads by soft tissues involves changes in structural protein conformation. This process was described in detail for a tendon. It was also confirmed that mechanical properties of soft tissues depend on collagen and elastin fibers orientation.

Keywords and phrases: Raman spectroscopy, collagen, tendon, aorta, soft tissues, mechanical properties, uniaxial tensile test.

Introduction

Collagen and elastin are fundamental proteins of human soft tissues, including tendons, ligaments, blood vessels and skin. Matrix components are responsible for their mechanical properties. Collagen fibres determine the strength while elastin fibres limit the elasticity. The quality and quantity of structural proteins are changed when tissues become dysfunctional. Biochemical analysis is essential for proper diagnosis. Moreover, the knowledge about soft tissue mechanobiology plays an important role in modern therapy, sport training and bioengineering [10, 13].

FT-Raman spectroscopy is a high potential technique for the non-invasive study of biological materials. This method provides information about the morphologic composition of tissues and can determinate small biochemical changes in their components, caused by diseases or other pathological processes [1, 6, 7]. Recent studies have shown that FT-Raman spectroscopy can be used for examination of the molecular deformation of natural materials under strain [2, 9, 13].

The aim of this study was to identify the Raman peaks which reflect the strain experimentally applied to the soft tissues. The spectroscopic results were compared with the mechanical relations obtained from the standard protocol of uniaxial tensile tests carried out in a testing machine. The correlation between the structure of soft tissues and their mechanical properties is discussed in the following article.

Methods

Materials

Tendon samples as an example of collagen-rich soft tissues were obtained from domestic pigs. Specimens of

human aortic walls were also studied. Samples of abdominal and thoracic aorta were excised in two directions: circumferential and longitudinal. Immediately after dissection the material was placed in saline (0,9% NaCl) and stored at temperature of 4°C. Raman spectroscopy and mechanical studies were performed.

Raman spectroscopy

FT-Raman spectra were recorded by an FT-Raman spectrometer Bruker RFS 100. A diode-pumped Nd:YAG laser at 1064 nm with an output of 450 mW was used as the excitation source. The Raman signal was detected by a Germanium detector. 128 scans were collected. The spectral resolution was 4 cm^{-1}. The reliability and precision of the wavenumber measurements was ≈ 1 cm^{-1}. The specimens were placed in a self-made tool designed specifically for the Raman scattering studies [5]. The tool was placed directly inside the spectrophotometer. Spectra were recorded for the successive phases of stretching the specimen (by 1 mm) to the maximum extension (22 mm) or sample rupture. The initial specimen length was always 28 mm.

Mechanical testing

Uniaxial tensile tests for equivalent specimens were performed using an MTS Synergie 100 testing machine on the day of harvesting. The initial specimen length was always 28 mm. The stress levels were observed with relation to applied strain.

Results and discussion

Representative Raman spectra of unstrained samples are shown in Fig. 1. For complex structures of human thoracic aortic wall band-rich spectra can be observed.

The major peaks in tissue spectra are attributed to proteins: $_{\delta}(CH_2,CH_3)$ (~1450 cm^{-1}), $_{\nu}(C_{\alpha}–C)$ (~940 cm^{-1}) and amide bands. The amide I vibration (1600--1700 cm^{-1}) is dominated by peptide carbonyl stretching vibration. Amide III (1200–1300 cm^{-1}) results from C–N stretching and N–H in-plane bending. Visible differences in the position and shape of amide bands are associated with various protein compositions of analyzed tissues. Amide I and III bands for triple helical structure of collagen are observed near 1668 and 1248 cm^{-1}, respectively. Elastin exhibits these bands in typical for unordered proteins positions: 1662 cm^{-1} and 1250 cm^{-1}. The bands near 877, 856 and 920 cm^{-1}, present in pig tail tendon spectrum, are assigned to $_{\nu}$(C–C) modes of amino acids characteristic for collagen: hydroxyproline and proline. Unique for elastin is desmosine, the presence of which can be clearly noticed in blood vessel spectrum (~1105 and ~1330 cm^{-1}). Apart from protein, one can also see lipid bands (1100–1150 cm^{-1}). It is also worth to notice that the Raman spectra of aortic wall in Fig. 1B show the presence of a weak band near 960 cm^{-1}, which can be attributed to the phosphate symmetric stretching mode of the hydroxyapatites deposited on the aortic wall as a result of the atherosclerotic process [3, 4, 6].

In the following paper the results obtained for Raman spectroscopy application in tendon measurements are mostly discussed. Tendon is considered as a model of rich-collagenous tissue (collagen represents 65–80% of the dry weight) [12]. It is worth mentioning that tendon has very hierarchical organization in which all structural units run parallel to the longest geometrical axis [8].

Characteristic regions are notable for a stress-strain curve for tendon. The stress values reach about 24 MPa. The value of the stress is connected with stress relaxation between stretching stages. However, to about 3–4% the stress-strain curve is linear. In the next steps the stretching stages generated less linear curve. After 10% of strain stretching stage is not fully completed and the yield point is crossed on the strain level about 15%. The first

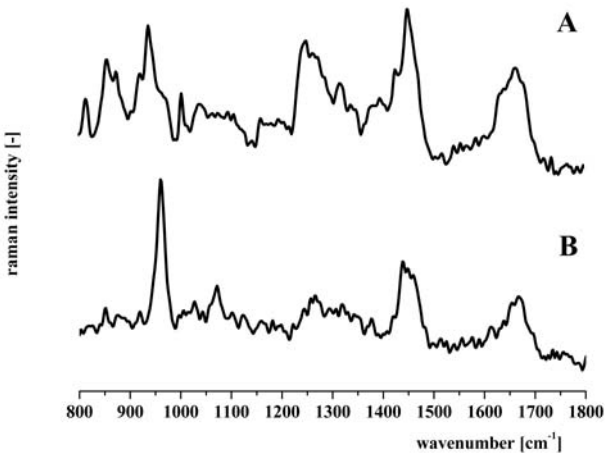

Fig. 1. Raman spectra of: A) pig tail tendon, B) human thoracic aortic wall.

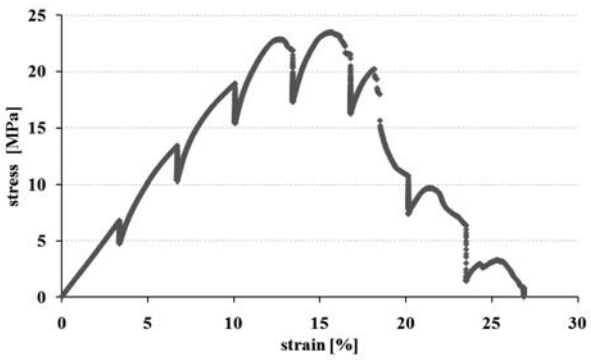

Fig. 2. Example of stress-strain curve for a tendon uniaxial tensile test. Peaks are typical stress relaxation connected with experimental protocol.

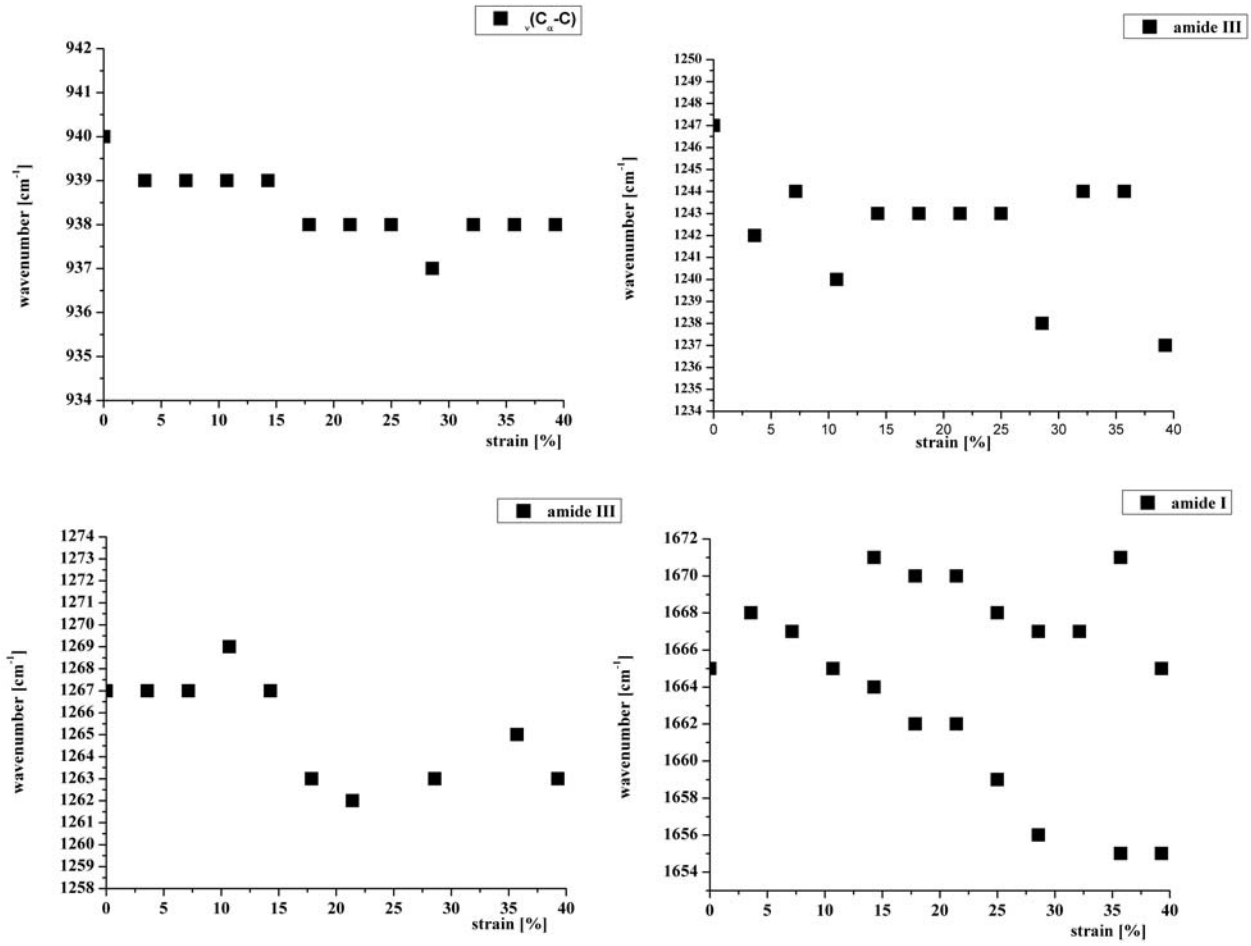

Fig. 3. Comparison of the component wavenumber for pig tail tendon as a function of the strain level.

signs of destroying the structure are notable on the level of 20–25%, when the stress is strongly decreased values.

The application of strain causes changes in Raman spectra and generates a stress in a sample (Fig. 3). The stress applied to chemical bonds leads to changes in interatomic distances and consequently, due to the anharmonicity of the vibrational energy, to changes in the position of the bands.

Noticeable amide bands alterations are noted. Peak positions explicitly change in stages. For Amide I (1665 cm^{-1}) few behavioural regimes can be distinguished. When the strain is in a range of 0–5% up shift occurs. At strain value higher than 10% doublet character of Amide I band becomes greatly visible. Later both components exhibits negative shift. In Amide III region in unstrained tendon spectrum two strong peaks are observed: band assigned to unordered (1248 cm^{-1}) and triplehelical structures (1267 cm^{-1}). This can be easily explained. In collagen molecule two structures are presents: proline rich fragments forming rigid triple helices and proline poor "loops" [8]. Originally loads are transferred by stretching unordered protein regions. Weak shoulder of amide III band (1242 cm^{-1}) probably origins from elastin, which is about 2% of the tendon dry weight. At strain value higher than 10% all protein is involved with loads transferring. A significant shift for C_α–C stretching vibrations at 938 cm^{-1} also took place. The observed decrease of the wavenumber value means that as protein backbone is elongating, molecular kinks are straightening. The band near 1004 cm^{-1}, assigned to the phenyl ring breathing mode of amino acid phenylalanine, as well as the modes assigned to $_\delta$(CH3) and $_\nu$(CH2), are not sensitive to the application of stress.

Fig. 4. Raman spectra of: A) human thoracic aortic wall, B) human abdominal aortic wall.

Figure 4 shows Raman spectra of two disease-free human arterial segments. Differences in amide bands are caused by differences in the protein composition: elastin predominates in the thoracic aorta (60%) and collagen is the main protein in the abdominal aorta (70%) [11]. The spectroscopic analysis also shows differing tension thresholds for material excised in two directions: circumferential and longitudinal. The amount and distribution of elastin and collagen fibres determine mechanical properties of the blood vessels. Uniaxial tensile tests of the aortic specimens confirmed that conclusion [5].

Summary

The mechanical behaviour of soft tissues depends on the amount of collagen and elastin fibres present and their distribution and orientation. Transferring loads by biological materials involves changes in structural protein conformation.

Acknowledgements

This work is supported by European Regional Development Fund and the Polish Government (Operational Programme — Innovative Economy) under the grant "WROVASC — Integrated Cardiovascular Centre" which is being realized in years 2007–2013.

References

[1] Beana, J.R., and B. Lendl. "Raman spectroscopy in chemical bioanalysis." *Current Opinion in Chemical Biology* 8 (2004): 534–539.

[2] Church, J.S., G.L. Corino, and A.L. Woodhead. "The effects of stretching on wool fibres as monitored by FT-Raman spectroscopy." *Journal of Molecular Structure* 440 (1998): 15–23.

[3] Debelle, L., et al. "The secondary structure and architecture of human elastin." *European Journal of Biochemistry* 258 (1998): 533–539.

[4] Dong, R., et al. "Temperature-dependent Raman spectra of collagen and DNA." *Spectrochimica Acta Part A* 60 (2004): 557–561.

[5] Hanuza, J., et al. "FT-Raman spectroscopic study of thoracic aortic wall subjected to uniaxial stress." *Journal of Raman Spectroscopy* 40 (2009).

[6] Manoharan, R., Y. Wang, and M.S. Feld. "Histochemical analysis of biological tissues using Raman spectroscopy." *Spectrochimica Acta A* 52 (1996): 215–249.

[7] Pappas, D., B.W. Smith, and J.D. Winefordner. "Raman spectroscopy in bioanalysis." *Talanta* 51 (2000): 131–144.

[8] Silver, F.H., J.W. Freeman, and G.P. Seehra. "Collagen sel-assembly and the development of tendon mechanical properties". *Journal of Biomechanics* 36 (2003): 1529–1553.

[9] Sirichaisit, J., R.J. Young, and F. Vollrath. "Molecular deformation in spider dragline silk subjected to stress." *Polymer* 41 (2000): 1223–1227.

[10] van Zuijlen, P.P.M., et al. "Collagen morphology in human skin and scar tissue: no adaptations in response to mechanical loading at joints." *Burns* 29 (2003): 423–431.

[11] Vouyouka, A.G., et al. "The role of type I collagen in aortic wall strength with a homotrimeric $[\alpha_1(I)]_3$ collagen mouse model". *Journal of Vascular Surgery* 33 (2001): 1263–1270.

[12] Wang, J.H.-C. "Mechanobiology of tendon." *Journal of Biomechanics* 39 (2006): 1563–1582.

[13] Wang, Y.-N., C. Galiotis, and D.L. Bader. "Determination of molecular changes in soft tissues under strain using laser Raman microscopy". *Journal of Biomechanics* 33 (2000), 483–486.

INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI
WOJEWODZKI SZPITAL SPECJALISTYCZNY WE WROCŁAWIU
WroVasc
UNIA EUROPEJSKA
EUROPEJSKI FUNDUSZ ROZWOJU REGIONALNEGO