THE USE OF NONLINEAR OPTICAL MEHTODS IN COMBINATION WITH TENSILE TESTING OF CONNECTIVE TISSUE IN RESPECT TO AGEING

[1,3]Daniel Hadraba, [1]Jiří Janáček, [3]František Lopot, [2]Eva Filová, [3]Ondřej Fanta, [3]Karel Jelen

1. Department of Biomathematics, Institute of Physiology of the ASCR, 142 20, Prague, Czech Republic
2. Institute of Experimental Medicine, ASCR, 142 20, Prague, Czech Republic
3. Department of Anatomy and Biomechanics, Charles University, Czech Republic
E-mail: dhadraba@gmail.com

SUMMARY

Medical approaches are increasingly orientated towards utilization of native and/or artificial tissue substitutes. The successful utilization of materials requires that they satisfy a set of properties [1]. One of the essential properties is chemico-mechanical uniformity of the substitutes compared to the original tissue. A sufficient insight into this issue, revealing the relationship between the mechanical properties and the structure, is vital. The combination of imaging methods and tensile tests is very beneficial and gives a strong insight into the origins of mechanical responses and the tissue functionality. The results are very favourable for a wide spectrum of applications (for example tissue engineering, composite engineering, drug delivery, etc.)

INTRODUCTION

According to current studies on tissue structure, the most mechanically influential segment overall is a protein matrix, the Extracellular matrix (ECM). The ECM is defined as a unique vico-elasto-plastic material. The elastic properties are established through proteins of final rigidity (preferably collagen and elastin fibres). The viscous character is mainly caused by the internal arrangement of proteins and inner fluid environment. As the ECM is a part of the open biological system that influences all processes in the human body (remodelling, regeneration, nutrition, etc.), it is highly probable that the mechanical properties are not stationary. This fact complicates detection of properties using the standard methods for material testing (tensile, torsion tests, etc.). Furthermore, the instability of the mechanical properties of tissue causes a significant error that is increased by the sample processing and finally it complicates the modelling and simulation of the whole tissue segment. This paper contributes to studying the combination of optical imaging methods and tensile tests. The possibility of correlation between visual data obtained thanks to nonlinear optical method of second harmonic generation (SHG) and polarised light microscopy and the mechanical response to loading discussed in respect to ageing offers new results that can answer some current questions about vico-elasto-plastic characteristics.

METHODS

Tendo calcaneus communis was dissected form the rabbits of different ages and the 20µm slices were visualised for type I collagen by backward nondescanned SHG Imaging (excitation at 860 nm by IR pulse laser, detection at 430±10 nm) at various depths up to 1500 µm; moreover, the excitation beam was polarisied at the angles of 0°, 60° and 120°. The received SHG pictures (2048 x 2048 pixels) were analysed for signal orientation in the way that 16 x 16 pixels were sampled with a step of 8 pixels. The structure tensor was calculated as a sum of structure tensors of the orientations (0°, 60°, 120°). To gain a colour coded picture (figure 1), each intensity ν and orientation angle φ was computed and expressed as a complex value in the pixel.

The absolute value codes the intensity in every pixel. After that the complex and real autocorrelation functions were calculated and the result was a decreasing function. Its mean value in the graph was used as a measure of the regularity of orientation.

Immediately after removing, the identical samples were also subjected to uniaxial tensile testing. The testing protocol consisted of both multiple cyclic loading and relaxation procedure. The force, elongation and time were recorded and analysed after executing the testing protocol.

RESULTS AND DISCUSSION

The results indicated that the regularity of type I collagen fibres was dependent on the age of the rabbits and showed a highly significant difference (p < .001) among the samples, however, the thickness and the number of fibres analysed in the histograms did not significantly (p = .05) vary among the different depths, ages of the samples.
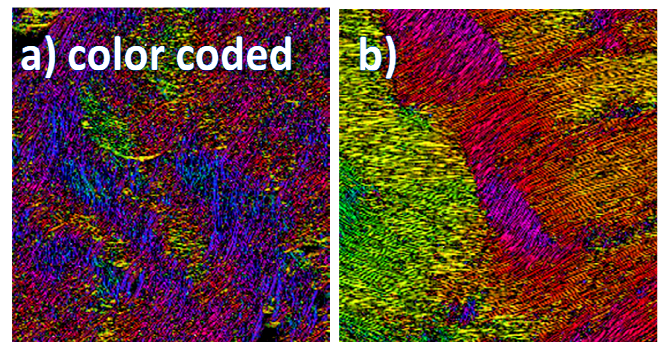

Figure 1: The colour coded pictures and type I collagen fibres: a) 4 week-old rabbit b) 9 month-old rabbit.

The tensile test curve representing the response to loading is particularly divided into two stages (figure 2).
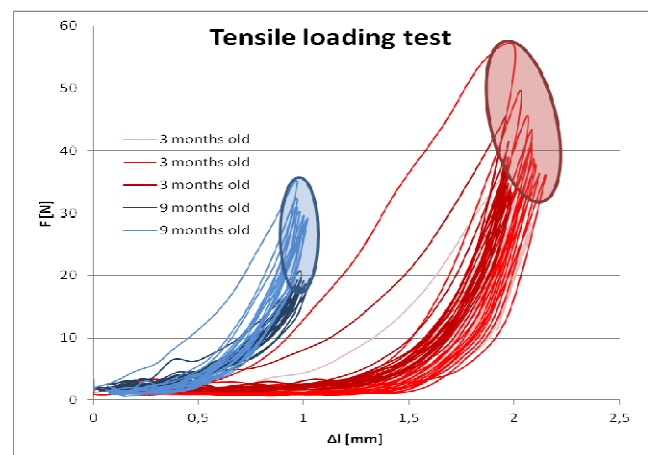

Figure 2: The cycling tensile test of 3 month-old rabbits and 9 month-old rabbits.

In its first stage, the stress reaction of the specimens to loading was minimal. In its second stage, the specimens displayed a response that was represented by the growth of the curve. When the curves were compared, it was evident that the curve of the younger samples (3 week-old rabbits) did not increase dramatically and furthermore it stayed about the same level. On the other hand the second stage of the curve was strongly growing while analysing the older specimens (9 month-old rabbits). The region of the shift, plastic deformation respectively, is indicated by the ovals (figure 2). The shift is much higher for the junior rabbits.

CONCLUSIONS

The aforementioned combination of both imaging and tensile methods is a powerful instrument that provides the information about the inner structure and mechanical behaviour of the whole system. In this study the older rabbits display higher level of regularity of the fibres and prompter response to loading. The results of combined techniques are very useful for example for comparing artificial tissue and native tissue directly from several points of view.

REFERENCES

1. Meyers M, Chawla K, *Mechanical Behavior of Materials.* Cambridge University Press, 808 pp. 2009.