

# ELASTIN CONTENT IN HUMAN INTERVERTEBRAL DISC – PRELIMINARY STUDY

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## Introduction

Elastin is an extracellular matrix protein which is most abundant in organs where elasticity is crucial, like blood vessels, lung or skin. Next to the collagen, elastin is involved in the process of load transfer and plays a key role in maintaining the appropriate mechanical properties of tissues [Daamen, 2007]. Insufficient literature data indicate that elastin content in intervertebral discs is at the level of 1÷2 percent [Smith, 2009]. Due to the lack of elastin in the intervertebral disc, arises the question about the function in this structure. Hence, main aim of this work is the quantitative and qualitative analysis of elastin in intervertebral discs in order to determine the cause of the presence of the protein in the tested structure.

## Methods

Elastin content in the human intervertebral discs were analyzed using immunohistochemical and immunoenzymatic ELISA methods.

ELISA method: Samples of the intervertebral discs were digested by trypsin and homogenized. MaxiSorp plates were coated with the tissue homogenates and standard solution of elastin. Three incubations were carried out: with 10% skim milk in TPBS, with the anti-elastin antibody and with ingredients of DAKO LSAB kit. Then OPD solution was applied and the absorbance at 450nm was read. Immunohistochemical method: Paraffin sections were deparaffined and incubated with proteinase K. The following reactions was conducted: with Real Peroxidase- Blocking Solution, Protein Block, anti-elastin antibodies, with the ingredients of DAKO LSAB kit + System-HRP and DAB. Hematoxylin was used as the counterstain.

## Results

The investigated human intervertebral disc samples (n = 5) contained an average 334µg elastin per 1g of wet weight of tissue, which gives only 0.033% of the tissue. Immunohistochemical investigations has

enabled a preliminary assessment of elastin arrangement in intervertebral discs (fig 1).

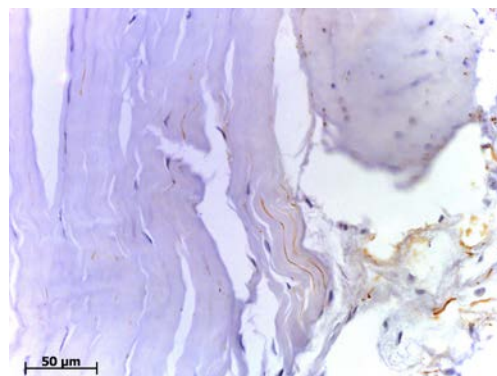


Figure 1: Elastin preserved in human intervertebral discs.

## Discussion

Presented here data are preliminary, however, show that the matched method allows to obtain reproducible results. It is not possible to directly compare the results obtained by the authors with the literature data. Elastin content of the total dry weight obtained by Mikawa [1986], Olczyk [1994] and Cloyd [2007] is respectively 1.7%; 1.5% and 2%. Presented data are significantly lower, because amount of elastin was analyzed in the wet tissue weight. However, the first immunohistochemical investigation revealed that arrangement of elastin fibers in intervertebral discs is consistent with previous authors (fig. 1).

## References

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