Type I collagen is a ubiquitous protein that is the main constituent of connective tissues. Degeneration processes influence the mechanical properties of tissue. The information obtained by different methods on the character and local mechanical properties of tissues could also serve as qualitative parameters of tissue for tissue engineers [1]. However, there is a need for non-invasive 3D imaging techniques to monitor and quantify collagen assembly in a 3D environment [2].

One of these techniques is a second harmonic generation (SHG) imaging, a non-invasive scanning optical method based on nonlinear properties of ordered non-centrosymmetric molecules (as collagen assembled into fibers) under extremely intense illumination. We have visualized the inner and outer parts of the human, cow and pig heart valves and endplates (part of the intervertebral disc), which are known to contain collagen. Simultaneously we have visualized the fluorescence of the cytoplasm of living cells labeled with CMFDA stain by a two-photon excitation microscopy (TPEM).

For mechanical characteristics of endplate we used a nano-indentation technique and combined these results with SHG imaging. The visualization of collagen by SHG in combination with nano-indentation in human heart valve and endplate has not been used yet. Precise knowledge of the structure of collagen fibrils in the inner and outer parts of the heart valve and endplate helps scientists to produce bioartificial heart valves and endplates of good quality to replace those currently used.

This work illustrates the potential of SHG microscopy for the characterization of collagen based structures in tissue engineering. As a result, we get unbiased information about the structural arrangement of inner and outer parts of human heart valve and endplate in combination with their mechanical properties which help to improve the design of materials used in tissue engineering.

The authors gratefully acknowledge funding from the Grant Agency of the Czech Republic under grant number P108/11/0794 and Grant Agency of the Ministry of Health CR, project No NT11270 – 4/2010, GAUK no. 545312 and institutional support RVO:67985823.