Connective Tissue: Diagnosis by AFM

We have applied atomic force microscopy (AFM) to diagnose morphological changes in the extracellular matrix of connective tissue caused by different pathological processes. Notable deviations from the normal morphology were found in the diseased tissues. The AFM data were found in a good agreement with the data of conventional histological studies. Thus, AFM may serve as an independent or complementary diagnostic tool for tracking pathological processes in the connective tissue.

AFM Bioimaging of Connective Tissue
Extracellular matrix (ECM) forms a basis of connective tissue, providing its specific mechanical properties. The ECM structure, and, particularly, the packing of collagen, the main protein ECM component, depends on the functional activity of cells and may significantly change in the presence of a pathological process.

Atomic force microscopy (AFM) has been currently widely used in the life science and biomedical studies [1]. The AFM studies of collagen-built structures, primarily, in the ECM of connective tissues have attracted a significant attention in the last years. A variety of connective tissue types have been probed by AFM and related techniques, including cartilage, tendon, intervertebral disk, skin, bone, tendon, vaginal wall tissue etc. (see, e.g., [2-6]). All these studies have demonstrated a high potential of AFM in tracking the collagen structures’ architecture in the connective tissue.

Tracking Connective Tissue Disease with Atomic Force Microscope
Here we have applied AFM imaging to monitor alterations in the ECM of connective tissue from different pathological causes, including 1) abnormalities of connective tissue leading to development of pelvic organ prolapse (POP) [6], 2) malignancy of connective tissue (chondrosarcoma), 3) radiation damage of the pelvic organs (bladder and rectum) resulting from radiation therapy. AFM imaging was performed in air on deparaffinized tissue sections, in the semi-contact mode.

Our AFM studies showed marked deviations from the normal ECM morphology of human skin and pelvic ligament for patients with POP [6]. The deviations were observed at all the levels of the ECM texture, including microtexture (packing of collagen fibers), nanotexture (arrangement of collagen fibrils) and structure of
individual collagen fibrils.

In particular, we observed visible separation, thinning and fragmentation of collagen fibrils and fibers, disintegration and disordering of collagen structures up to the complete destruction of the specific tissue architecture (fig. 1, left panel). The nanoindentation study revealed significant deterioration of the mechanical properties of the collagen fibrils bundles in the skin of POP patients, as compared to the skin of healthy subjects (fig. 1, right panel).

In the AFM study of bone tumor tissue, we compared the morphology of chondrosarcoma of histologically malignant grade I, II and III. A benign chondroma tumor was used as a control. The AFM imaging showed a clear correlation between the content of the fibrous collagenous elements in the ECM of a bone tumor and the degree of its malignancy (fig. 2). While the ECM of chondroma and grade I chondrosarcoma were represented mostly by the network of collagen fibrils, the grade II chondrosarcoma contained a substantial fraction of non-fibrous elements, and AFM images of the ECM of the grade III chondrosarcoma showed only the non-fibrous amorphous material.

The AFM study of the ECM of internal pelvic organs damaged by radiation therapy have shown the first ECM changes occur 1 week post-irradiation in the dose of 2 Gy. 1 day after the radiation treatment, no differences with the intact tissue were detected. 1 week post-irradiation, the normal basket-weave architecture of collagen fibers and fibrils (fig. 3, 1A-1C) in the ECM of the bladder connective tissue transformed into a fine mesh covered by a non-fibrous unstructured protein (fig. 3, 2A-2C), possibly resulting from inflammation. No ECM alterations were detected for the rectum at this time point. 1 month post-irradiation, both bladder and rectum ECM showed clear signs of fibrosis manifested as thick bundles of collagen with parallel stacks of fibrils inside (fig. 3, 3A-3C).
The obtained AFM data demonstrated a good agreement with the conventional histological study data for the POP study and chondrosarcoma study. For the radiation damage study, the histological data did not reveal any significant alterations of the bladder and rectum’s morphology, independently of the time passed after the irradiation. Thus, the AFM sensitivity exceeded that of a conventional histological technique in the latter case.

**Conclusions**
The obtained data confirm that AFM may be considered as a promising independent or complementary technique for the nanodiagnostics of connective tissue disease. The future development should be focused on the quantification of the AFM morphology data.

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**References**

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More information on AFM: http://www.imaging-git.com/tags/atomic-force-microscopy