Extracellular matrix (EM) forms a basis of human connective tissue, providing its specific mechanical properties. The EM structure, and, particularly, the packing of collagen, the main protein EM component, depends on the functional activity of cells and may significantly change in the presence of a pathological process. Here we have applied atomic force microscopy (AFM) to diagnose morphological changes in the EM of connective tissue caused by two different pathological processes – connective tissue dysplasia leading to pelvic organ prolapse (POP) and a neoplastic process (tumors of bone). AFM imaging was performed on air on deparaffinized tissue sections. Our AFM studies showed marked deviations from the normal EM morphology of human skin and pelvic ligament for patients with POP. The deviations were observed at all the levels of the EM 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). 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.

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 EM of a bone tumor and the degree of its malignancy (Fig.2). While the EM 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 EM of the grade III chondrosarcoma showed only the non-fibrous amorphous material. The AFM data on the EM structure of normal and pathologically altered connective tissue were found being in a good agreement with the data of the standard morphological methods (including histological and electron microscopy analysis) on the same clinical specimens. Thus, AFM and related techniques may serve as either an independent, or a complementary diagnostic tool for tracking pathological changes in the connective tissue.

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Fig. 1: AFM-diagnostics of pelvic organ prolapse (POP). The loss of order in the collagen fibrils packing in the extracellular matrix of the skin of POP patients (A-C) as compared to normal skin (D). The scan sizes are 3x3 µm.

Fig. 2: AFM-diagnostics of the bone tumors’ degree of malignancy. The fraction of fibrous collagenous elements of the extracellular matrix decreases with the malignancy grade. C – chondroma (benign tumor); CS1, CS2, CS2 – I, II and III grade chondrosarcoma, respectively. The scan sizes are 3x3 µm.