Tissues, Pathology, and Diagnostic Microscopy

LS.2.P038
Nanodiagnostics of pelvic organ prolapse based on the atomic force microscopy analysis of extracellular matrix

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Keywords: atomic force microscopy, extracellular matrix, pelvic organ prolapse

Extracellular matrix, consisting mainly of collagen, forms a basis of human connective tissue, providing its specific mechanical properties. The supramolecular packing of collagen macromolecules results in a characteristic nano- and microtexture for each type of connective tissue (skin, ligament, intervertebral disc etc.). Pathological processes may lead to significant changes in the structure of extracellular matrix of connective tissue, and, in particular, in the collagen packing.

Here we have applied atomic force microscopy (AFM) to diagnose pathological changes in the extracellular matrix of connective tissue caused by pelvic organ prolapse (POP). POP is a common condition affecting women which considerably decreases the patients' quality of life [1].

We have studied clinical specimens from skin biopsy of patients with POP and those without connective tissue disorders (control group). AFM imaging was performed on air on deparaffinized tissue sections.

AFM images of normal human skin demonstrated a characteristic "basket-weave" pattern of collagen fibers (Figure 1, *a*) [2], consisting mostly of tightly packed quasi-parallel collagen fibrils (Figure 1, *b* and *c*), with occasionally found disordered regions. The specific banding of collagen fibrils (D-period) was clearly resolved, especially in the phase images. The details of collagen molecular packing could be visualized at a high resolution, with the "gap" (corresponding to 4 parallel collagen molecules in the collagen microfibril) and the "overlap" (5 collagen molecules in the microfibril) regions forming together the D-period of collagen [3], and their finer structure. Besides the collagen bundles, fibers without banding (presumably, elastic fibers) and regions with high deformability and adhesion (apparently, non-fibrous components of the extracellular matrix) were found.

For the specimens taken from the POP patients, we detected significant deviations from the normal skin structure. The "basket-weave" pattern of collagen bundles became coarser, with the holes visibly expanded (Figure 2, *a*). The increased deformability and probe adhesion to the surface testified increase in the fraction of non-collagenous components of the extracellular matrix. In contrast to the normal skin morphology, we only rarely observed the tight quasi-parallel packing of collagen fibrils, while the disordered weaving of separate fibrils prevailed (Figure 2, *b* and *c*). Within the tightly packed regions, we often found deformation, rupture and shortening of collagen fibrils, as well as decrease in the average fibril thickness comparing to the normal skin from the control group.

The hardness and Young's modulus of the bundles of collagen fibrils measured by nanoindentation appeared to be considerably lower for the POP tissue samples as compared to those for the control group. Thus, both the morphological and mechanical data have been found to be meaningful in the potential POP diagnostics.

The AFM data were compared to the data of standard clinical morphological studies (including histological and electron microscopy studies) of the same specimens. According to the traditional methods, similar signs of deterioration of the normal extracellular matrix structure were found for the POP patients' tissue, including visible separation of collagen fibers, thinning and fragmentation of collagen fibers, disintegration and disordering of collagen structures up to the complete destruction of the specific dermis architecture. The consistency of the AFM findings and the results of the standard morphological methods points out to the validity of the AFM analysis as a diagnostic tool for the POP-related structural changes of extracellular matrix.

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4. We kindly acknowledge the financial support from the Russian Foundation for Basic Research (Grant #12-02-00633-a).

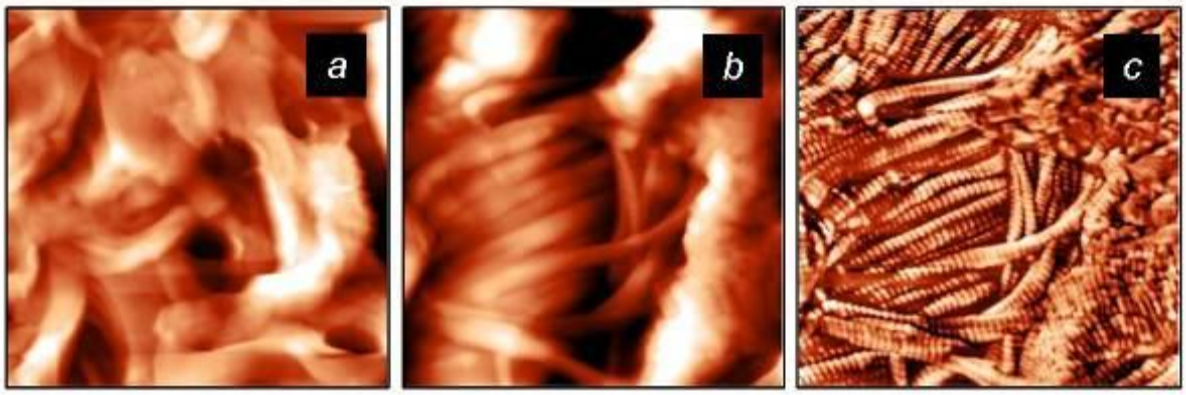

Figure 1. Micro- (*a*) and nanotexture (*b, c*) of extracellular matrix of normal human skin.
a – topography, scan size is 14×14 μm, height scale is 0 - 4.0 μm.
b – topography, *c* – phase, scan size is 3×3 μm, height scale is 0 – 500 nm.

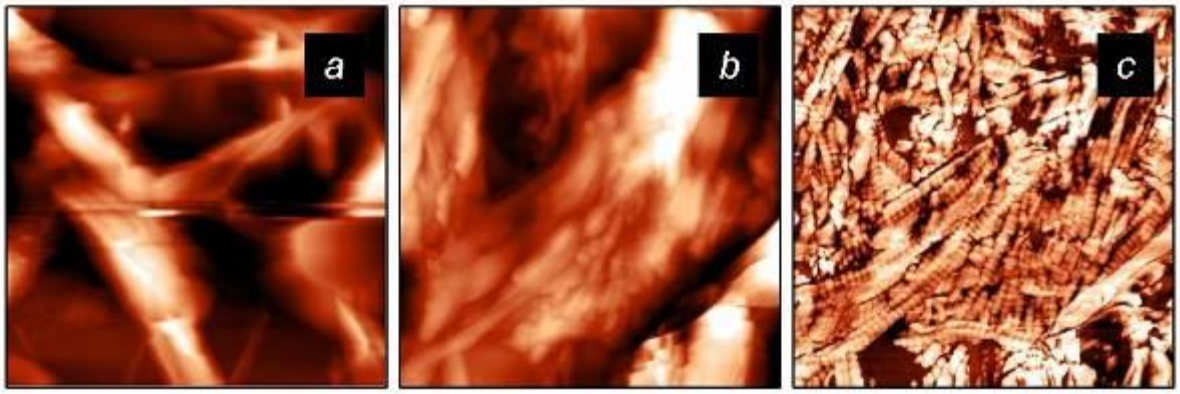

Figure 2. Micro- (*a*) and nanotexture (*b, c*) of extracellular matrix of the skin of a patient with POP.
a – topography, scan size is 14×14 μm, height scale is 0 - 4.0 μm.;
b – topography, *c* – phase, scan size is 3×3 μm, height scale is 0 – 500 nm.
Note the inferior quality of Image *a* resulting from the deep holes, material softness and high adhesion to the probe.