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Atomic force microscopy: a promising aid in diagnosis of uterine smooth muscle neoplasms

Leonardo A. Peñuela, MD, PhD, Valerio G. Vellone, MD, PhD, Gian M. Vigliercio, MD, Roberto Fiocca, MD, Ezio Fulcheri, MD, Roberto Raiteri, EE, PhD, Simone Ferrero, MD, PhD

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RESEARCH LETTER

1. TITLE:

**Atomic force microscopy: a promising aid in diagnosis of uterine smooth muscle neoplasms**

2. AUTHORS:

Leonardo A. PEÑUELA, MD, PhD; Valerio G. VELLONE, MD, PhD; Gian M. VIGLIERCIO, MD; Roberto FIOCCA, MD; Ezio FULCHERI, MD; Roberto RAITERI, EE, PhD; Simone FERRERO MD, PhD

From the Fetal-Placental and Perinatal Unit, Giannina Gaslini Institute, DiNOGMI, University of Genoa, Genoa, Italy (Drs. Peñuela and Fulcheri); Pathology Unit, San Martino Hospital, DISC, University of Genoa, Genoa, Italy (Drs. Peñuela, Vellone, and Fiocca); Medicine School, University of Genoa, Genoa, Italy (Dr. Vigliercio); Department of Biomedical Engineering, Robotics and Systems (DIBRIS), University of Genoa, Genoa, Italy (Dr. Raiteri); and Department of Gynecology and Obstetrics, DiNOGMI, University of Genoa, Genoa, Italy (Dr. Ferrero)

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5. CORRESPONDING AUTHORS:
    a. Valerio Gaetano Vellone MD, PhD, (valerio.vellone@unige.it)
    b. Leonardo Alett Peñuela MD, PhD, (leonardopenuela@outlook.com)

    Department of Pathology, Policlinico IRCCS San Martino-IST, Via De Toni, 14, 16132, Genova, Italy, Phone: (+39) 010 555 5921.

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OBJECTIVE:

Malignancy diagnosis of uterine smooth muscle neoplasms can be challenging. Morphological features are subjective, and utility of immunohistochemistry is still debated.[1,2] We propose to characterize and compare the ultrastructural and mechanical properties of leiomyoma and leiomyosarcoma with those of normal myometrium, using atomic force microscopy (AFM) technique.

STUDY DESIGN:

Three representative groups of samples were selected from the database of the Pathology Unit–San Martino Hospital, Genoa (Italy). **Group 1**: one sample of *normal myometrium* and one sample of spindle cells *leiomyoma* from a 39-year-old patient, and one sample of spindle cells *leiomyosarcoma* from a 62-year-old patient. **Group 2**: one sample of *normal myometrium* and one sample of spindle cells *leiomyoma* from a 49-year-old patient, and one sample of spindle cells *leiomyosarcoma* from a 67-year-old patient. **Group 3**: one sample of *normal myometrium* and one sample of spindle cells *leiomyoma* from a 50-year-old patient, and one sample of spindle cells *leiomyosarcoma* from a 55-year-old patient.

Two shadowed sections (thickness of 5µm for AFM-imaging and 15µm for AFM-indentation testing) were separately collected from each paraffin-embedded tissue specimen. After paraffin removal and drying, the specimens were left exposed for AFM-scanning. Sections were not stained.

AFM-imaging was performed in contact mode at room temperature and in air on regions of interest (ROIs), which were accurately selected through optical microscopy. The specimens for mechanical characterization were probed in liquid conservation medium (PBS) at room temperature through AFM-nanoindentation on three 32x32-array/90x90$\mu m^2$ ROIs. Image processing was made using

Fiji (https://doi.org/10.1038/nmeth.2019). Elastic modulus values, calculated from AFM-indentation test, were statistically analyzed ($p \leq 0,05$) using MedCalc Software v.18.11 (Ostend, Belgium).

RESULTS:

AFM-imaging evidences regularly oriented smooth muscle cells in *normal myometrium*. The nuclear pattern shows blunt-ended, thin ellipsoidal nuclei, centrally located in sarcoplasm. Larger myofibrils and smaller connective fibrils show similar positioning, bestowing high homogeneity to the interstitium (Figure 1A). Similarities in regular orientation of smooth muscle cells, nuclei density, shape, and location in the sarcoplasm, were observed between *Leiomyoma* and *normal myometrium*. However, despite such similar directionality, myofibrils are narrower and the interstitium is comparatively wider and devoid of small connective fibrils (Figure 1B). Contrarily, *leiomyosarcoma* evidences irregularly oriented pleomorphic smooth muscle cells. Nuclei appear bigger (>50% of sarcoplasm), more rounded and at a higher density than in *leiomyoma* and *normal myometrium*. Interstitium shows further loss of myofibrils and small connective fibrils, that are replaced by amorphous, non-fibrillar material. This feature confers high irregularity and heterogeneity to the tissue ultrastructure (Figure 1C).

Concerning the AFM-indentation measurements, performed on each group of samples, ANOVA-test indicates statistically significant differences between the elastic moduli of different samples ($p<0,0001$), and *t*-test showed a significantly lower average elastic modulus (i.e. softer tissue) for *leiomyosarcoma* when compared to *leiomyoma* and, an even lower average elastic modulus, when compared to *normal myometrium* (Figure 1D). Interestingly, nearly 80% of elastic modulus values measured on different points of leiomyoma, and over 95% of those measured in leiomyosarcoma, are below the mean elastic value $\bar{E}$ of the normal myometrium (Figure 1E).

CONCLUSION:

*Leiomyoma*, *leiomyosarcoma*, and *normal myometrium* show significant differences at the nanoscale, that cannot be easily observed and measured at the microscale with clinically available techniques. Ultrastructural differences in myofibrils positioning and thickness, nuclear pattern, and interstitium are clearly observed. Similar to other tumor types, elastic modulus of tumorous myometrium decreases compared to normal myometrium.[3–5]

Based on these preliminary results, we propose AFM as a technique capable of providing complementary and useful morpho-mechanical parameters, in terms of differential diagnosis in gynecopathology.

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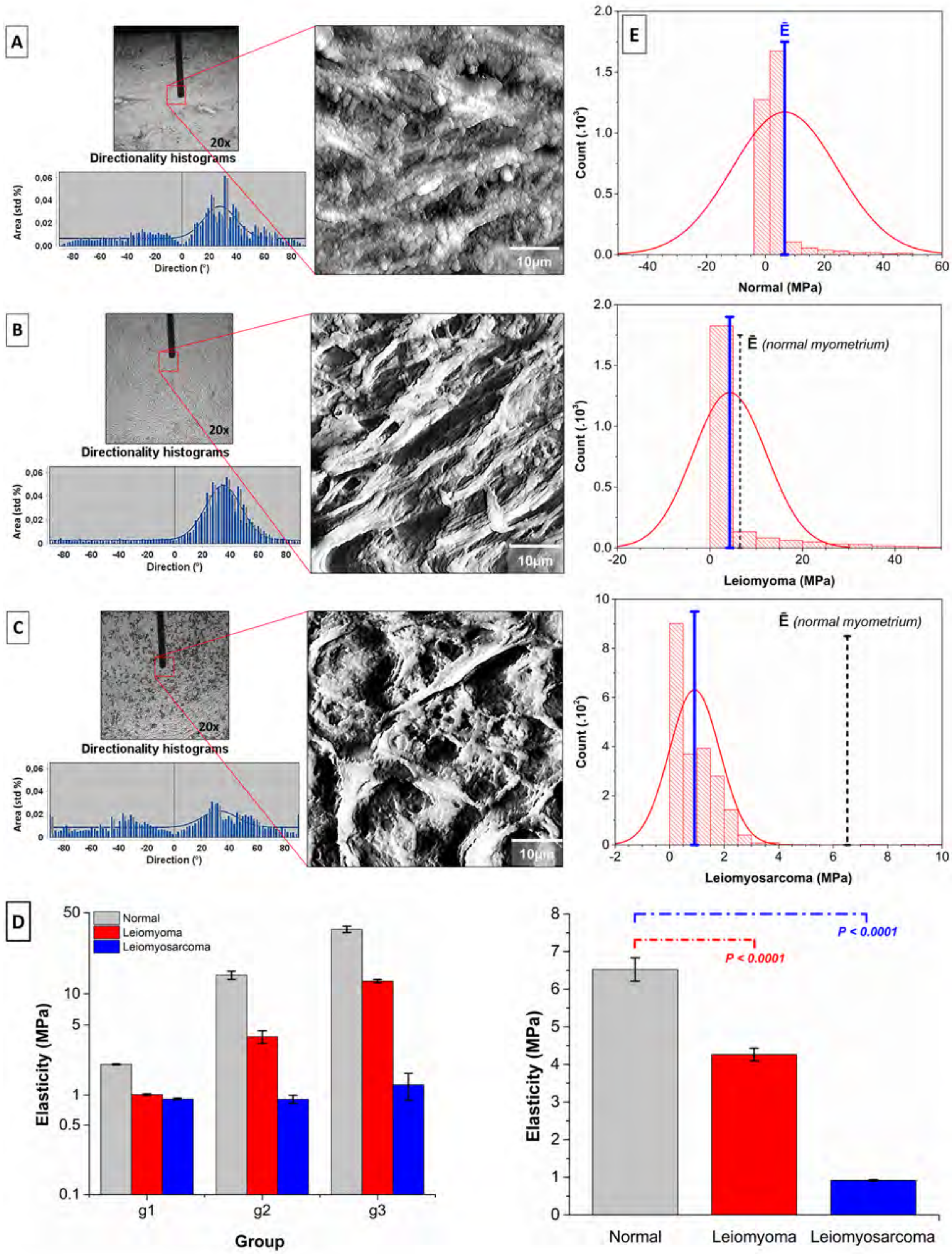
FIGURE LEGENDS

**Figure 1. Atomic force microscopy ultrastructural and mechanical characterization.**

**A**. *Normal myometrium*; **B**. *Leiomyoma*; **C**. *Leiomyosarcoma*: **Left column**, optical microscopy images where the red squares are the selected ROIs for AFM-scanning; histograms of the preferential direction calculated for the corresponding AFM image; **right column**, representative 50x50µm$^2$ AFM topography images of the selected ROI. **D**. **Left plot**: average elastic modulus calculated from AFM-nanoindentation measurements on each sample of groups; **right plot**: cumulative average elastic modulus of groups classified by type of tissue, (error bars: 1SEM). **E**. **1$^{st}$ to 3$^{rd}$ plots**: Distribution of the measured elastic moduli for each sample; $\bar{E}$ represents the average elastic modulus of the *normal myometrium*.



A
20x
Directionality histograms
Area (std %)
Direction (°)
10µm
B
20x
Directionality histograms
Area (std %)
Direction (°)
10µm
C
20x
Directionality histograms
Area (std %)
Direction (°)
10µm
E
Ē
Count (.10³)
Normal (MPa)
Ē (normal myometrium)
Count (.10³)
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Leiomyosarcoma