Taking nanomedicine teaching into practice with atomic force microscopy and force spectroscopy

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Atomic force microscopy (AFM) is a useful and powerful tool to study molecular interactions applied to nanomedicine. The aim of the present study was to implement a hands-on atomic AFM course for graduated biosciences and medical students. The course comprises two distinct practical sessions, where students get in touch with the use of an atomic force microscope by performing AFM scanning images of human blood cells and force spectroscopy measurements of the fibrinogen-platelet interaction. Since the beginning of this course, in 2008, the overall rating by the students was 4.7 (out of 5), meaning a good to excellent evaluation. Students were very enthusiastic and produced high-quality AFM images and force spectroscopy data. The implementation of the hands-on AFM course was a success, giving to the students the opportunity of contact with a technique that has a wide variety of applications on the nanomedicine field. In the near future, nanomedicine will have remarkable implications in medicine regarding the definition, diagnosis, and treatment of different diseases. AFM enables students to observe single molecule interactions, enabling the understanding of molecular mechanisms of different physiological and pathological processes at the nanoscale level. Therefore, the introduction of nanomedicine courses in bioscience and medical school curricula is essential.

atomic force microscopy; hands-on course; human blood cells; teaching nanomedicine

NANOMEDICINE is an emerging scientific field that has progressively become highly recognized by the entire research community. Nanomedicine can be defined as the application of nanotechnologies in medicine using molecular knowledge of the human organism to maintain and improve human health at the molecular scale (36). Nanotechnology plays a role in the creation and development of devices for the analysis of living systems at the “nano” level. Fundamental aspects in this context are the design, characterization, production, and application of structures that have novel physical, chemical, and biological properties at the nanoscale level (44).

Nanotechnology is revolutionizing medicine in the 21st century. However, only very few people receive specific training in both medicine and nanotechnology or how nanotechnology is likely to impact new medical products, drug discovery and delivery, or the practice of medicine using nanotechnology (20).

In the last few years, there has been an increased effort to attract the interest of clinicians for the use of the knowledge and technology generated by nanomedicine researchers. There is a general acceptance that nanomedicine could rapidly increase its impact by informing, educating, and updating people who every day deal with human disease on nanomedicine-based developments to monitor, diagnose, and cure diseases (5, 26, 27, 36). Safety considerations, public awareness of what is feasibly possible and close contact with reality, as well as the assessment of the needs of the clinicians who will use the nanomedicine tools are also aspects that should be taken into account by nanotechnology researchers (43). Most of the large pharmaceutical groups are nowadays still weakly attracted to the concepts and technologies that nanomedicine presents, often because they still consider them risky and expensive (26, 27).

In 2002, the United States National Nanotechnology Initiative, proposed by the National Science Foundation, began a program to promote activities for different levels of student education in nanosciences (41). The education challenge still is to bridge disciplinary and geographical barriers, developing a new framework to reverse the education pyramid from general knowledge to specialization. Major research universities in the United States have begun to implement multidisciplinary programs in nanosciences, involving physical sciences, life sciences, and engineering (5, 47, 50). In 2004, education in nanotechnology has also been defined as a priority at the European Union level, but only recently nanoscience education became part of curricula in several universities and high schools in Europe (22, 28).

A state-of-the-art technique that is convenient to use for the teaching of nanomedicine and nanotechnology-based techniques is atomic force microscopy (AFM). Atomic force microscopes are one of the most powerful tools developed at this level. AFM is mainly an imaging technique, in which the surface of a sample is scanned, line by line, by the movement of a thin tip mounted on a flexible cantilever (for a review, see Ref. 42). The tip-sample repulsion at the atomic level, transduced by the cantilever deflection and by an optical lever mechanism, allows the association of a height value to each position on the x,y plane and, therefore, the reconstitution of a high-resolution pseudo three-dimensional image of the sample surface.

Besides imaging, another major application of AFM is to quantify the interaction between the tip and a sample, taking advantage of the pico-Newton sensitivity of the method. This approach is usually termed “force spectroscopy” (despite not being in fact a “spectroscopy,” as it does not involve the interaction of radiation with matter) (6, 10, 35). By measuring the variations of the force exerted on the sample, AFM enables the detection of specific interaction forces at the single molecule level. The possibility of modifying the surface and manipulating individual molecules made AFM an ideal tool for biological and biomedical applications (9, 10, 16, 29, 33).
Putting into practice the objectives regarding nanomedicine education established in the United States and Europe, the main goal of our work was to introduce nanomedicine, specifically the application of AFM, on the study of biomedical systems to Doctor of Medicine (MD) and Doctor of Philosophy (PhD) students from the Lisbon Academic Medical Centre (which brings together the Medical School of the University of Lisbon, Institute of Molecular Medicine, Santa Maria Hospital, and Pulido Valente Hospital). In particular, we presented a teaching model of AFM as a new imaging and functional diagnostic tool. We focused on imaging human blood cells and a specific example for the interaction between a plasma protein (fibrinogen) and its specific receptor on platelets (glycoprotein-\(\alpha_{IIb}\beta_{3}\)).

The physiological process of blood clotting and the subsequent dissolution of the clot, after repair of the injured tissue, are essential components of blood hemostasis. When a blood vessel is damaged, platelets prevent the occurrence of extensive bleeding, with the formation of a dense fibrin network, leading to blood clotting (31). Platelet membrane glycoproteins specialized in receptor-ligand recognition are important in the response to a vascular wall injury (37, 38). Fibrinogen, one of the most abundant plasma proteins, is essential for blood clotting. It polymerizes, forming a fibrin network that entraps erythrocytes and platelets to form a clot. Fibrinogen, also known as coagulation factor I, is a soluble glycoprotein and is mainly synthesized in the liver by parenchymal cells but also by platelets (24). Fibrinogen contains three potential integrin-binding sites (two arginine-glycine-aspartic acid amino acid sequences within the \(\alpha_{IIb}\)-chain and a 12-amino acid (dodecapeptide) sequence on the \(\gamma\)-chain) but can also interact with cells through nonintegrin receptors (24). Platelet membrane glycoprotein complex-\(\alpha_{IIb}\beta_{3}\) is the well-characterized platelet integrin receptor for fibrinogen (4). Ligand binding to this integrin heterodimer involves specific regions of the \(\alpha_{IIb}\)-terminal portions of both \(\alpha_{IIb}\) and \(\beta_{3}\) units (30, 40).

The importance of understanding the structure-function relationship of biomolecules cannot be overstressed. AFM may play a crucial role for those studies, contributing to the nanomedicine knowledge that is required for fruitful developments in nanodiagnostics and nanotherapeutics, contributing to the improvement of relevant healthcare processes. The strategy presented here proved to be a successful introduction to nanomedicine for MD and PhD students.

**MATERIALS AND METHODS**

**Course structure.** This advanced training course was implemented for the first time in 2008, enrolling the students of the PhD Program of Lisbon Academic Medical Centre. The European Credit Transfer and Accumulation System-credited course is designed for 15 PhD students divided into groups of 3–4. These students come from different basic formations, such as medicine, biochemistry, or biology. PhD students attending this course are mainly from the first and second years of their doctoral training.

The course allows students to gain hands-on experience with an atomic force microscope. At the beginning of the course, the tutor gives an introduction on AFM, including its different potentialities and limitations. Students have also the opportunity to hear about and discuss different applications with researchers active in different areas of nanomedicine research invited to give a seminar about their research. This close contact between work and education is especially important in cutting-edge subjects such as nanomedicine. After this introductory component, students have the opportunity to shift from theory to hands-on practice. Practical sessions commence with the AFM imaging of biomedical samples followed by a second session on force spectroscopy measurements of protein-cell receptor interactions. Each session takes ~2 h.

A feedback form for students to fill at the end of the course was also implemented. The questionnaire assesses the contribution of the course to the increase of awareness and knowledge of students on AFM and also evaluates how the course changes student perceptions of the potential applications of this technique in the nanomedicine field.

**Human blood cell isolation and deposition.** Human blood cells were isolated as previously described elsewhere (7). Blood was collected from adult healthy donors into K₂EDTA anticoagulant tubes, following their previous written informed consent, as approved by the joint Ethical Committee of Santa Maria Hospital and the Faculty of Medicine of the University of Lisbon. Platelets were isolated from platelet-rich plasma (PRP). Briefly, PRP was separated by centrifugation at 220 g for 7 min at 10°C. Platelets were pelleted at 1,620 g for 10 min and washed three times with buffered saline-glucose-citrate (BSGC) buffer (1.6 mM KH₂PO₄, 8.6 mM Na₂HPO₄, 0.12 M NaCl, 13.6 mM sodium citrate, and 11.1 mM glucose) pH 7.3 with CaCl₂ (1 mM). The final pellet suspension was resuspended in the same buffer and kept at 4°C before use. Platelets were counted using a Cell-Dyn 1600 (Abbott).

To prevent transitory platelet activation during the isolation protocol, prostacyclin can be added to the platelet suspension. Prostacyclin is usually used at each step of the platelet washing procedure at a final concentration of 0.5 μM. Alternatively, platelets can be isolated in the presence of iloprost, a stable prostacyclin analog (11).

For the AFM imaging session, a blood smear was prepared on a glass microscope slide with a drop of fresh human blood and left to dry at room conditions for 30 min. For the force spectroscopy hands-on experiments, platelets were used at a concentration of 10,000 cells/μl. Five hundred microliters of the platelet suspension were placed on a clean poly-L-lysine-coated glass slide surface and allowed to deposit for 30 min. Nonadherent cells were removed by five sequential dilutions with BSGC buffer with CaCl₂ (1 mM), loaded into the atomic force microscope, and allowed to equilibrate in the buffer for 15 min before force spectroscopy measurements.

**AFM imaging of human blood cells.** A NanoWizard II atomic force microscope (JPK Instruments) mounted on the top of an Axiovert 200 inverted optical microscope (Carl Zeiss) was used for imaging and force spectroscopy experiments. The AFM head is equipped with a 15-μm z-range linearized piezoelectric scanner and an infrared laser. Imaging of the blood smear was performed in air, in tapping mode. Oxidized sharpened silicon tips with a tip radius of ~6 nm, resonant frequency of 60 kHz, and spring constant of 3 N/m were used for the imaging. Imaging parameters were adjusted to minimize the force applied on the scanning of the topography of the cells. Scanning speed was optimized to 0.3 Hz, and acquisition points were 512 × 512. Imaging data were analyzed with JPK image processing version 4.2.61 (JPK Instruments).

**AFM tip functionalization and force spectroscopy measurements.** Force spectroscopy measurements were performed on the same equipment using fibrinogen functionalized OMCL TR-400-type silicon nitride tips (Olympus). The softest triangular cantilevers, with a tip radius of ~15 nm and a resonant frequency of 11 kHz, were used. For the functionalization, AFM silicon nitride tips were cleaned with an intense ultraviolet light source and silanized in a vacuum chamber with 3-aminopropyl-triethoxysilane (30 μl) and N,N-diisopropylethylamine (10 μl) for 1 h in an argon atmosphere. Amine-terminated AFM probes were then placed in glutaraldehyde solution [2.5% (vol/vol)] for 20 min and washed three times with BSGC buffer with CaCl₂ (1 mM). Finally, the tips were placed in fibrinogen solution to attach the fibrinogen molecules. Purified human fibrinogen (Sigma) was used at a concentration of 1 mg/ml with a 30-min incubation (3, ...
Fibrinogen-functionalized tips were immediately mounted on the atomic force microscope and used for the force spectroscopy practical sessions.

RESULTS

**AFM imaging.** Through the practical sessions, it was possible to collect high-resolution AFM images of the human blood smears. Some of them are shown in Fig. 1, where different types of blood cells can be observed. In Fig. 1A, the majority of the imaged blood components are erythrocytes, with their characteristic biconcave shape. In the top left corner of the image, two echinocytes can also be seen. This is a different, less frequent, erythrocyte morphology, in which the erythrocyte is crenated, with numerous, uniform spicules throughout the membrane-cytoskeleton complex (32), as shown in Fig. 1A. In the bottom left corner of the same image, a leukocyte can also be observed, with some black dots characteristic of granular leukocytes (neutrophils, basophils, and eosinophils). These granules (specific and azurophilic granules) contain certain enzymes and other proteins that have the function of neutralizing or destroying invading microorganisms (15). In this image, we can also observe the differences in height between erythrocytes and leukocytes. Erythrocytes are much thicker (brighter colors) than leukocytes. In Fig. 1B, the surface and morphology of blood cells (from a different sample) are imaged with a higher magnification. The multilobed nucleus of a leukocyte can be clearly seen. Based on the observed morphology, this cell can be identified as a neutrophil, a polymorphonuclear leukocyte. The ability of this technique to image not only surface features but also intracellular components is therefore clearly demonstrated. This degree of detail is possible because there are differences in rigidity among intracellular components in height at the surface of the neutrophil between the membrane on the top of the nucleus and around it (32).

Another possibility highlighted in these sessions is the ability of AFM to measure differences of the cells’ surface roughness, which can be clearly seen in Fig. 1B. From Fig. 1B, one can qualitatively conclude that neutrophils have a higher cell surface roughness than erythrocytes. To quantify this parameter, specific mathematical models have to be applied (1, 18, 48), but this was out of the scope of the training session.

Another tool to quantitatively analyze AFM height images is to measure sizes on the samples (human blood cells in this case) and their surface profile. To perform this, cross sections of the cells were analyzed, yielding the two distinct

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Fig. 1. Atomic force microscopy (AFM) scanning images of blood smears. Air tapping-mode AFM height images of human blood cells from healthy donors are shown. A: AFM image of different human blood cells (horizontal scale: 50 × 50 μm²; height scale up to 1.48 μm). Most of the imaged cells are typical circular, biconcave erythrocytes. There are also some echinocytes, especially on the top left corner of the image, and a leukocyte in the bottom left corner. The black dots in its cytoplasm are specific and/or azurophilic granules characteristic of these cells. B: human blood cells at a higher magnification (horizontal scale: 25 × 25 μm²; height scale up to 1.25 μm). A large neutrophil is visualized at the center of the image, with its characteristic multilobed nucleus. Around this cell, some biconcave erythrocytes are also seen. C: cross-sectional analysis of the AFM image (left), with two examples of the cell profiles (right). With this type of analysis, cell size, height, shape, and roughness can be determined.
profiles shown in Fig. 1C. This analysis indicates that the neutrophil is 13.2 μm in diameter, with a maximum height of 440 nm. With the cross-sectional profiles of both cells, one can also differentiate the roughness of the cell’s surface and the biconcave shape of the erythrocyte. The analyzed erythrocyte is 8.9 μm in diameter and has a height of 759 nm on the border of the cell and 595 nm on its concavity.

**Force spectroscopy.** Force spectroscopy measurements were done with an AFM tip functionalized with fibrinogen molecules (covalent attachment of the protein) interacting with platelets deposited on a poly-l-lysine coated glass slide. All the force spectroscopy principles and settings that are necessary to be controlled to perform these measurements were briefly explained by the tutor of the sessions, such as applied force, velocity of the tip, dwell times, z-axis length, the use of the thermal fluctuation noise method to calibrate the cantilevers, and the different force spectroscopy operation modes. Applied force is defined by the indentation force or upward force applied by the AFM tip upon contact with the sample as a function of the deflection (bending) of the cantilever. More adhesion events are usually observed upon increasing the applied force. The velocity of the tip is the rate of change of the cantilever deflection (in nm/V) and the value of its spring constant (in N/m) to obtain force (in N) by applying the well-known Hooke’s law (46).

After some approach-retraction cycles have been performed, force-distance curves such as those shown in Fig. 2 are obtained. The force-distance cycle starts with the tip far away from the cell surface, with the cantilever on its neutral position (no bending, 0-pN force). Upon moving down toward the surface (red curves in Fig. 2), the tip reaches the contact point, and blue lines represent the retraction curves of approach-retraction cycles. A: force-distance curves with no detectable adhesion forces. B: a fibrinogen-platelet membrane receptor single unbinding event. The cycle starts with the tip far away from the cell surface (e), with the cantilever on its neutral position (0-pN force, no bending), and starts moving down toward the sample surface. Upon reaching the contact point (f), the cantilever starts bending upward, indenting the surface, until a defined trigger force is reached and the movement of the tip stops (g). After a waiting time, the tip begins an upward movement away from the sample, reaching the contact/adhesion point (h), where the force is again 0 pN. If no tip-sample adhesion occurs, the tip continues its upward movements, at 0 pN of force (A). If a binding occurs between fibrinogen and its cell membrane receptor, as the cantilever moves upward, it will bend down, yielding a peak of negative force values (i) in the force-distance plot. Upon reaching a given force value, resulting from the cantilever deflection, the fibrinogen-platelet bond breaks and the cantilever jumps back to its neutral position (j), completing the force-distance cycle.

**Fig. 2.** Typical force-distance curves obtained by AFM-based force spectroscopy for the fibrinogen-platelet interaction. Red lines represent the approach curves, and blue lines represent the retraction curves of approach-retraction cycles. A: force-distance curves with no detectable adhesion forces. B: a fibrinogen-platelet binding event.

For quantitative force measurement, the spring constant of the cantilever must be first calibrated, so that the z-axis displacement (distance) of the deflection of the cantilever can be converted into a force value. There are different ways to calibrate spring constants of cantilevers (21, 49); one of them is the thermal fluctuation noise. This is the most accurate method for calibrating cantilevers in fluid. It was first introduced by Hutter and Bechhoefer in 1993, who quantified the frequency and amplitude of thermal fluctuations of the cantilever (25). The amplitude of these fluctuations for a given temperature depends only on the spring constant of the cantilever. It is also necessary to calculate the value of the deflection of the cantilever, which is determined by the ratio between the distances that the cantilever deflects for a certain measured change in photodetector voltage. If one knows the cantilever deflection (in nm/V) and the value of its spring constant (k), then it is straightforward to convert values of deflection (in V) to force units. The deflection (in m) is multiplied by the spring constant of the cantilever (in N/m) to obtain force (in N) by applying the well-known Hooke’s law (46).
where the cantilever starts bending upward and indenting the sample surface until a defined trigger force is reached and the approach stops. The waiting time before the start of the retraction curve is usually called the dwell time. Upon retraction (blue curves in Fig. 2), the tip, indented on the cell surface, begins upward movements away from the sample, reaching the contact/adhesion point, where the force is again 0 pN (no bending). If no binding adhesion occurs between the fibrinogen molecules attached to the tip and the cell surface, the tip continues its upward movement at 0 pN (Fig. 2A). If an adhesion between the cell and fibrinogen occurs, as the cantilever moves upward it bends down, yielding negative force values in the force-distance plot (Fig. 2B). In some surface distance points, the bond between fibrinogen and the cell breaks and the cantilever returns to its neutral position, completing an approach-retraction cycle.

AFM measurements for imaging or force spectroscopy can be done in contact mode, where the tip is always “touching” the sample, or in oscillating modes, such as the intermittent-contact (or tapping) mode and noncontact (or close-contact) mode. Upon using the intermittent-contact mode for force spectroscopy, the system locates the surface using amplitude modulation and only stops the tip oscillation during acquisition of a curve. Noncontact force microscopy is able to detect small long-range forces acting between the AFM tip and sample surface (13, 17). Both are less frequently used in force spectroscopy because the resulting force-distance curves are difficult to interpret and analyze.

Data collected from the force-distance curves allowed the determination of the rupture force, rupture length, and binding frequency between fibrinogen and its platelet membrane receptor. Rupture force is defined as the force necessary to break the bond between one molecule of fibrinogen and one cell receptor, which is characterized by the instantaneous jumps in force shown in Fig. 2B,i. Rupture length values are defined as the distance (in nm) between the cell-AFM tip contact point (defined as the zero-length value; Fig. 2B,j) and the height at the moment of the breaking of the bond between fibrinogen and the cell receptor. Binding frequency (or the probability of binding) is defined as the number of single molecule adhesion events (>10 pN of force in the present study) per total number of contacts between fibrinogen molecules and cell receptors (total number of approach-retraction curves acquired).

Students are expected to analyze the force curves obtained (~1,500 curves in this study) using specific software for analysis of AFM images and curves. In our laboratory, they could use JPK image processing software (version 4.2.61), but other software packages, such as Gwydion or Scanning Probe Image Processor could also be applied. Some of them can be freely downloaded on the internet (e.g., Gwydion). Data are analyzed and adjusted by polynomial fit, and histograms of the unbinding forces could be constructed choosing the ideal bin size to achieve the best fitted Gaussian model peak forces. From each histogram, the single fibrinogen molecule rupture force can be determined by fitting the distributions of the rupture forces with the Gaussian model.

The results were in accordance with those obtained on the measurements with platelets carried out for the sake of comparison in parallel with the study of the binding between fibrinogen and its recently discovered receptor on erythrocyte membranes (7, 8).

The presence of prostacyclin when performing the platelet isolation preserves their resting (nonactivated) state. This procedure should be carried out when force spectroscopy experiments are intended to be conducted with platelets in a state as close as possible with the physiological conditions.

It is also important to highlight to the students the broad range of biological and biomedical applications of AFM-based force spectroscopy (2, 35) and how to obtain the necessary information from the force-distance curves. Based on the data acquired from the approach curves on force spectroscopy measurements, it is possible to perform indentation and stiffness (surface elasticity) studies. Based on the retraction curves, information on the unfolding/folding of proteins, ligand-receptor binding, inhibitor binding, cell-cell adhesion, molecular adhesion, and protein-cell receptor adhesion studies can be carried out (9, 23, 29, 45).

Evaluation. At the end of each course, a brief evaluation survey was given to students to allow them to provide their feedback on the course. In the questionnaire, students were asked to give a score based on a 1 ("bad") to 5 ("excellent") scale for each part of the hands-on sessions. Additionally, students gave an overall rating to the course and could also provide comments or suggestions for upcoming courses. The results (n = 23) showed an overall rating of the course of 4.7, which means a score between "good" (4) and "excellent" (5) and closer to the latter. For the questions on the general understanding of the course and if the course was useful, both analyses yielded average scores of 4.5. The highly positive feedback from students suggests that, in general, they were very satisfied with the course. The questionnaire also aimed to understanding how the students first learned about the existence of the course. The different responses were distributed between the Medical School of the University of Lisbon webpage, the Institute of Molecular Medicine webpage, from a colleague, by specific direct e-mail, and by their own PhD supervisors. Through the hands-on sessions, all students were very enthusiastic, asking plenty of questions and participating in every activity proposed.

**DISCUSSION**

Here, we present a model for the teaching of one of the diverse nanomedicine components at the university graduation level. The course was designed for PhD students, and the good evaluation results allowed us to conclude that its implementation was done with full success. For the majority of students that participated on the course, this was their first contact with an atomic force microscope and with the technique. At the end of the session, students gave very positive feedback to the instructor. Several students left the room thinking and discussing with the instructor how they could apply this technique on their specific projects. Other students would like to learn more about the technique and made specific suggestions at the end of the questionnaire asking for more time for the practical sessions on future courses. There were no negative comments provided by the students. All comments will be taken into consideration on the preparation of future courses. In these courses, the number of students interested in this field and on attending to the course was considerably above the stipulated limit (15 students/course). Due to the high level of interest, in the future the course will be open to more participants.
The methodology used to evaluate this course proved to be extremely useful. The introduction of a end-of term questionaire allowed us to perform an analysis of student learning and analysis of student feedback (12, 19, 34). The first summarizes the strengths and weakness of a student’s learning and indicates how the course could be revised to enhanced future performance. The feedback of the students indicates how to change the course or teaching in response to student concerns. The nature and organization of the course often impact student perceptions of it. Thus, after analyzing their feedback, we concluded that the introduction of this course was successful.

With the proposed practical sessions, we showed that AFM is not only a powerful experimental tool but also a very influential educational model to introduce specific aspects of nanomedicine to graduate students. One of the many advantages of the experiments proposed is that their preparation is very simple and rapid and has a low operation cost. For each course, we only used four AFM cantilevers (two for AFM imaging and two for the force spectroscopy measurements). Students also left the practical sessions with a good perspective of a broad range of biological and biomedical applications of the AFM technique. Therefore, this course can be implemented in other universities with access to an atomic force microscope and to small blood samples (not necessarily human). The explanation of the AFM experiments was kept as simple as possible, so as to be accessible to MD and PhD students with different backgrounds.

AFM can be used to give students a better understanding of the intermolecular or intramolecular mechanisms associated with many diseases. The practical sessions of this course can bridge the gap between the nanoworld (atomic and molecular levels) and the macroworld (the phenomena). The use of this nanotechnology is extremely important to assist researchers at the submicron level. Understanding the molecular basis of disease affects the practice of medicine from future medical students. We thus believe that, with this course, students are more alert to the use of AFM as a powerful scientific tool and also as an educational tool. The use of AFM for nanotechnology education at other universities has already been proven to be successful (6, 39, 47). However, to the best of our knowledge, this is the first time in which the training was not restricted to imaging, further extending to force spectroscopy measurements.

We believe that, in the near future, nanomedicine will have amazing and far-reaching implications in medicine regarding the definition, diagnosis, and treatment of different diseases. Thus, it is urgent to adopt significant and effective measures to introduce nanomedicine courses in medical school curricula. The good assessments of the course led us to carry on with it for new PhD students as well as to MD students. We think that the broadening of the knowledge in this field of undergraduate students, graduate students, and young scientists may have an extremely important positive impact on future daily good research and medical practice.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

F.A.C. and N.C.S. conception and design of research; F.A.C. and T.F. performed experiments; F.A.C. and T.F. analyzed data; F.A.C. and N.C.S. interpreted results of experiments; F.A.C. prepared figures; F.A.C. drafted manuscript; F.A.C. and N.C.S. edited and revised manuscript; F.A.C. and N.C.S. approved final version of manuscript.

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