SHAPE- A New Theoretical Framework of the Microgravity-Cell Interaction

S. Bellucci (Resp. Naz.), S.Bistarelli (Dott.), A. Cataldo (Bors.), M. Mastrucci (Laur.), F. Micciulla (A.R.),

External collaborating Institutions:

Dipartimento di Ingegneria Elettronica dell'Università di Roma Tor Vergata, Italy
Dipartimento di Medicina Sperimentale della Seconda Università degli Studi di Napoli, Italy
Università "Gabriele D'Annunzio" di Chieti e Pescara, Italy
Project Consulting S.r.l.Roma, Italy
Istituto Superiore di Sanità, Roma, Italy

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Aims of the Project

Defining the theoretical model that can explain the interaction between the gravitational field and living organisms. The theoretical framework of this report is not only a prerequisite for the development of the Space Biomedicine and Exobiology, but is likely to have important consequences on the Biology and Medicine study, to the extent that allows to reconsider its foundations in the role played by physical constraints in the control of processes and vital functions of complex living systems.

Investigating the extent to which the effect induced by microgravity on living structures is dose-dependent and within what time limits can be defined reversible.

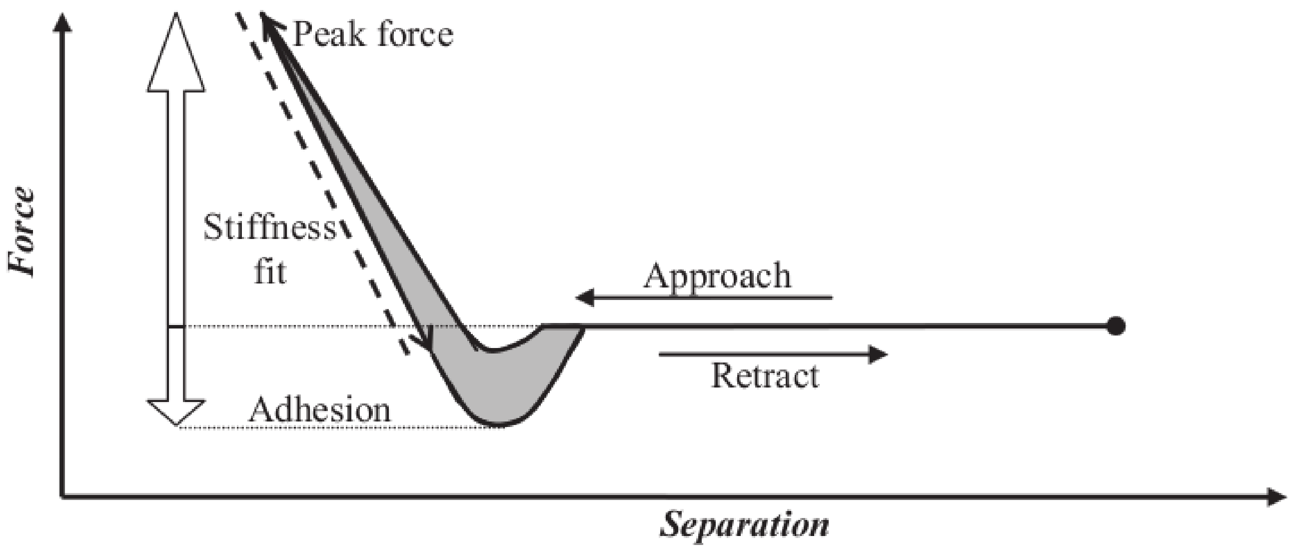
Refining the experimental model and scientific methodology inherent in the study of microgravity. In particular, it proposes to validate a new experimental protocol for the study of gravitational effects in 3D. This aspect is of fundamental importance since the control and the determination phenotypic evolution cell and tissue depend not only from the cell itself, but also and mainly by interactions with the stroma and the structures of the micro-environment. This cross-talk between the cell and the micro-environment in three dimensions is not so far never been investigated in the specific conditions represented by microgravity.

Preliminary data suggest that the microgravity weakens (reduced stiffness) the interactions between cells and substrate. Because such interactions are largely modulated by the cell class of integrins (proteins that modulate the form ("SHAPE") and anchor it to the stroma), and considering that their integrity is ensured by a heterogeneous class of molecules (melatonin, procyanidins, epigallocatechin etc ..), we propose that, in the context of countermeasures such substances are preliminarily tested to assess the extent to counteract the effects of the reduction of gravity.

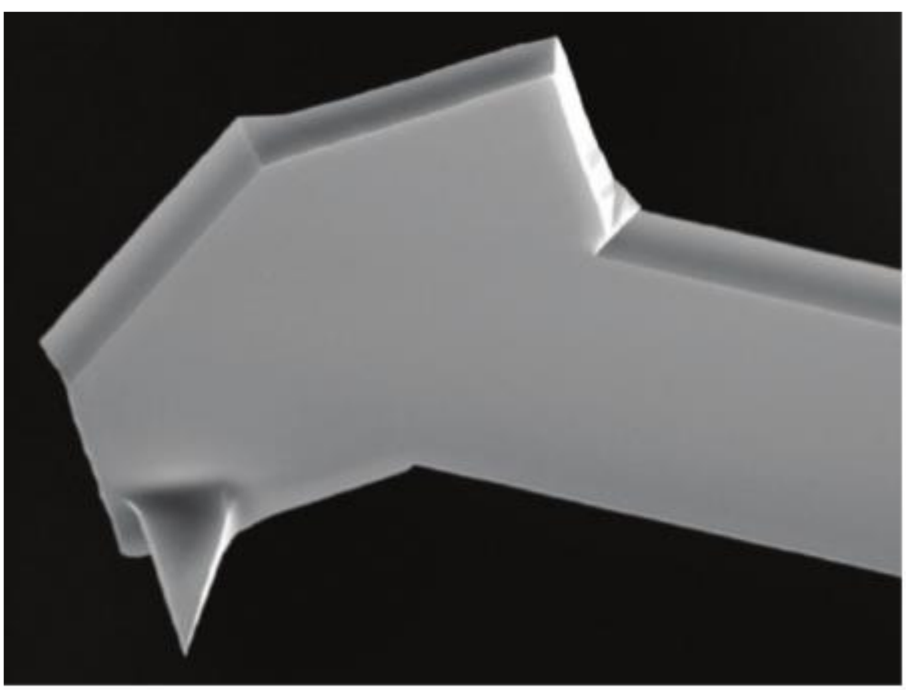
It is also proposed to take preparatory measures to believe the experiment on the ISS, using bioreactors already available and / or under construction. This will involve the preparation of a draft appropriately spatialized study.

Research Activity

In order to understand the interaction between gravity and matter Atomic Force Microscope was used. It interacts directly with the surface of the biological sample without any special preparation. It allows us to get information about topography, surface rigidity, adhesion to the substrate and elasticity (see Fig 1a) [1]. All these data compared and correlated with those from other characterization techniques and analysis allow to verify which changes can occur after exposure to microgravity (comparing them with a control sample) and seek to understand whether these effects are reversible or irreversible.



(a)



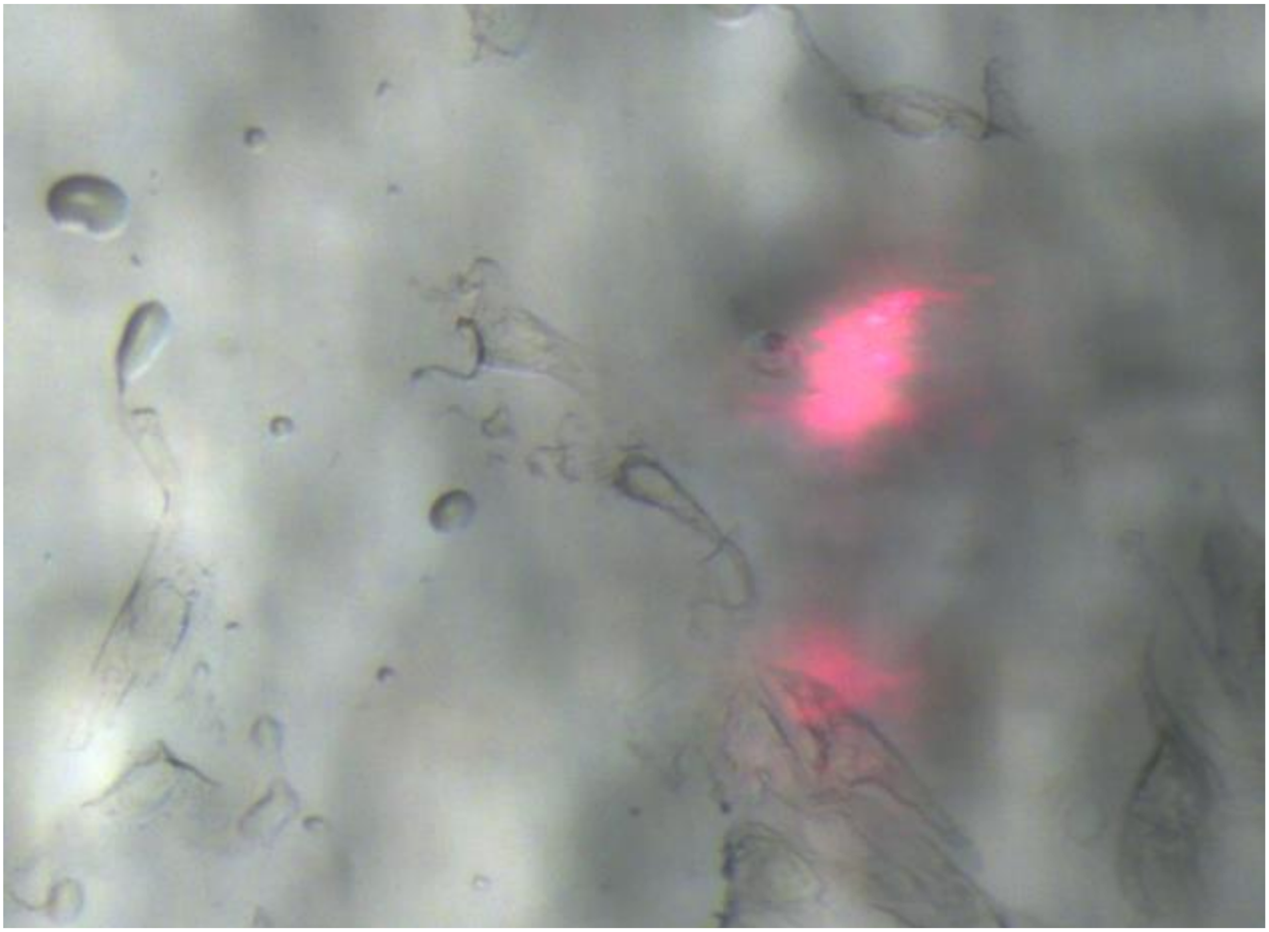
(b)

Figure 1: (a) Force vs. separation curve. (b) HarmoniX cantilevel shows an asymmetrical connected tip.

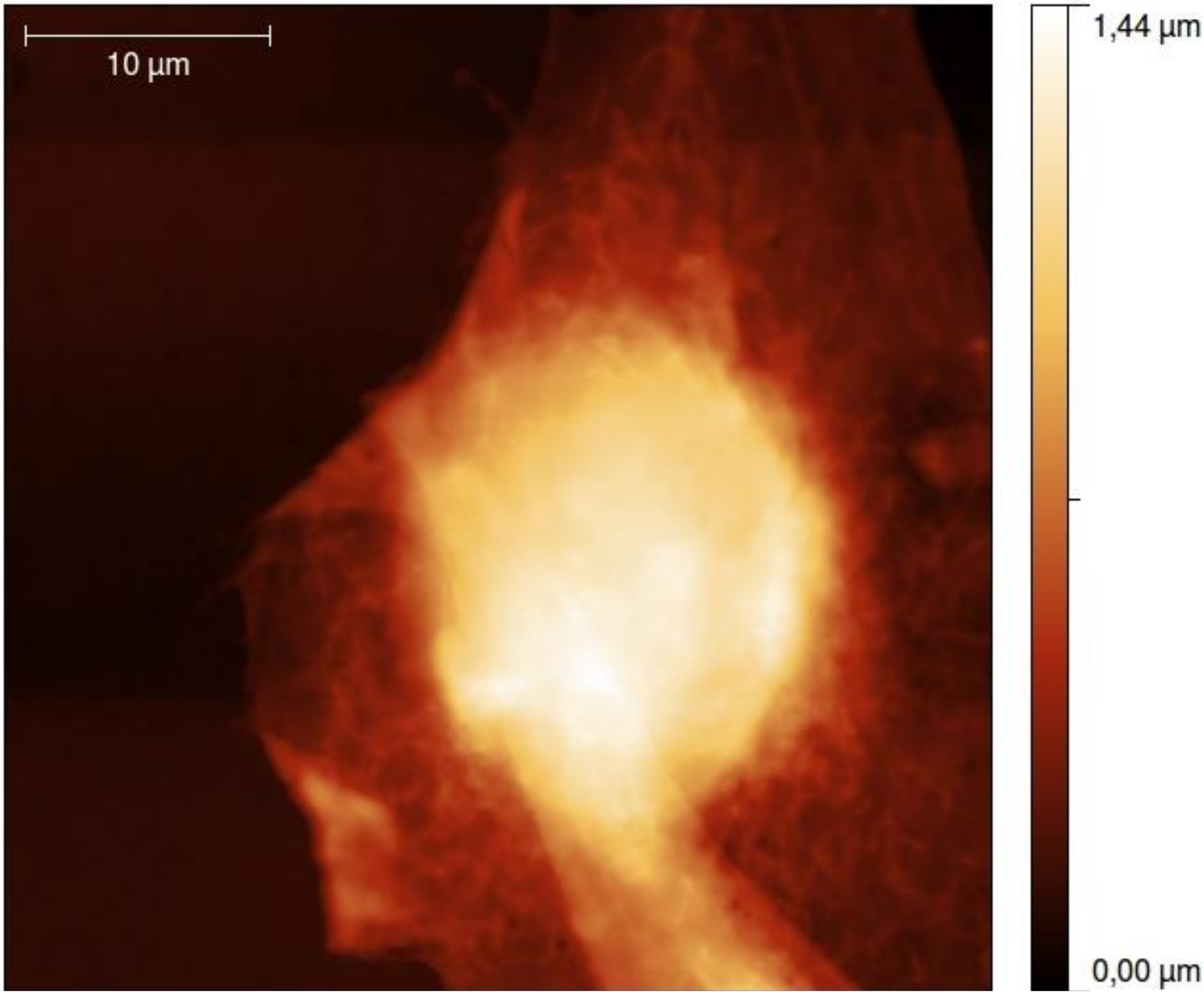
Atomic Force Microscopy (AFM) measurements were performed in Tapping Mode by using a Dimension Icon microscope (Brucker AXS, Germany), simultaneously recording topographic and mechanical properties images for every analyzed sample. For HarmoniX mapping torsional harmonic cantilevers (HarmoniX Probes, HMX-10, Veeco Instruments Probes, Camarillo, CA) (resonance frequency 60 kHz, spring constant 4 N/m) were used (Fig 1b).
HarmoniX measurements were done in air under ambient conditions using NanoScope version 7.30 software (Veeco/Digital Instruments, Santa Barbara, CA). For DMT modulus determination the cantilevers were calibrated using a standard PS/LDPE sample[2].
HarmoniX Tapping Mode AFM was operated utilizing cantilever vibration free amplitude of 1.5 V in air. The level of the force applied to the surface was adjusted by the amplitude setpoint, which was used for the feedback control, to ca. 40% of the free amplitude. Imaging was performed at 0.5Hz scan rates. Image processing and data analysis were performed with the Gwyddion software version 8.10, and NanoScope Analysis software version 1.10.
Before examination with AFM, fixed cells were washed three times with solution containing glucose (0.1M) and NaCl (0.1M) with ratio 2:1.

<u>Topography</u>

Before the examination of the mechanical properties we qualitative analyzed the morphology of the different cells. MC3T3 cells are used in this work. These are osteoblast precursor cell lines derived from mouse and widely used as model systems in bone biology.
Firstly we analyzed the untreated samples. In Figs. 2 and 3 we can see some examples of the cells.



(a)



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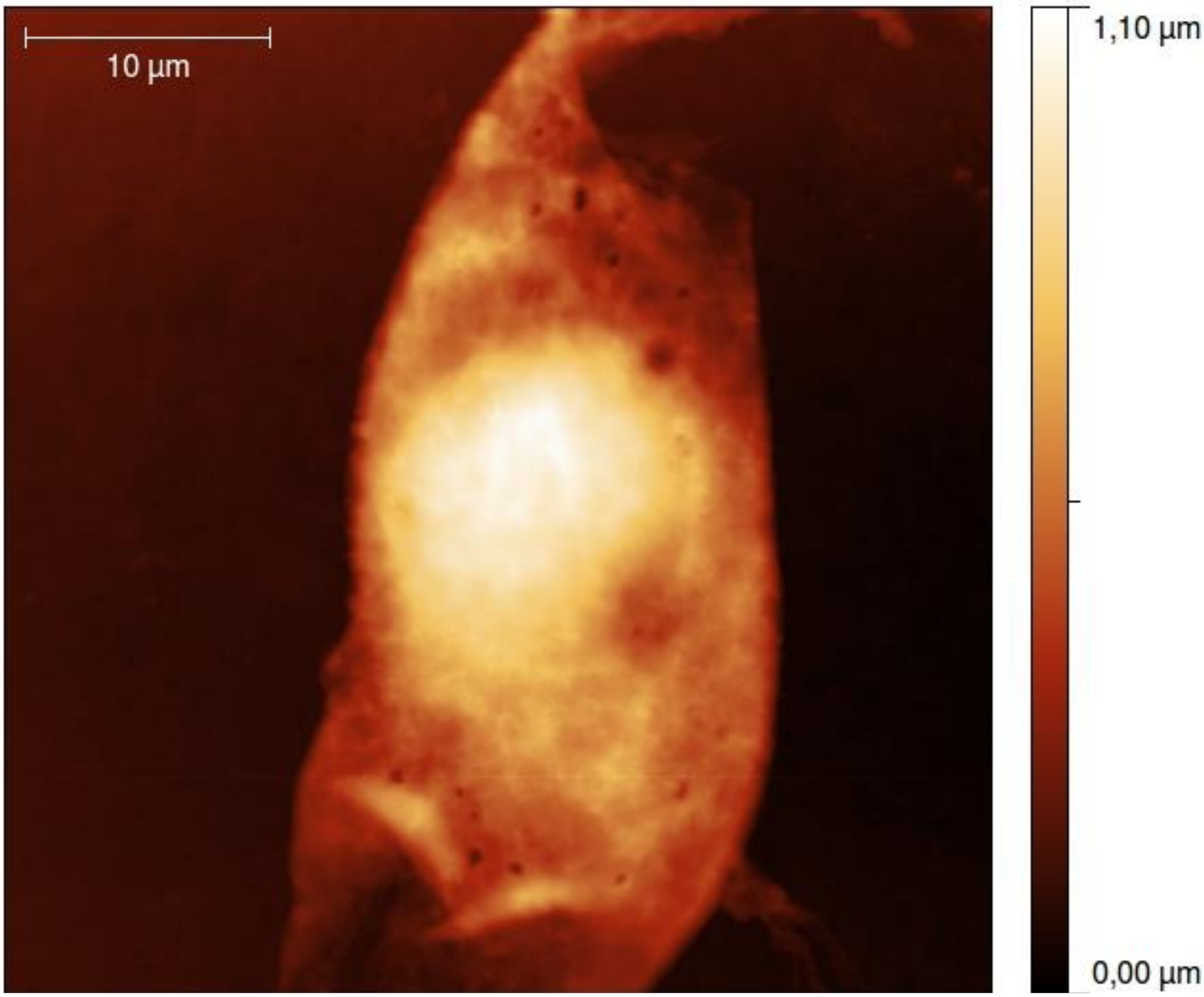
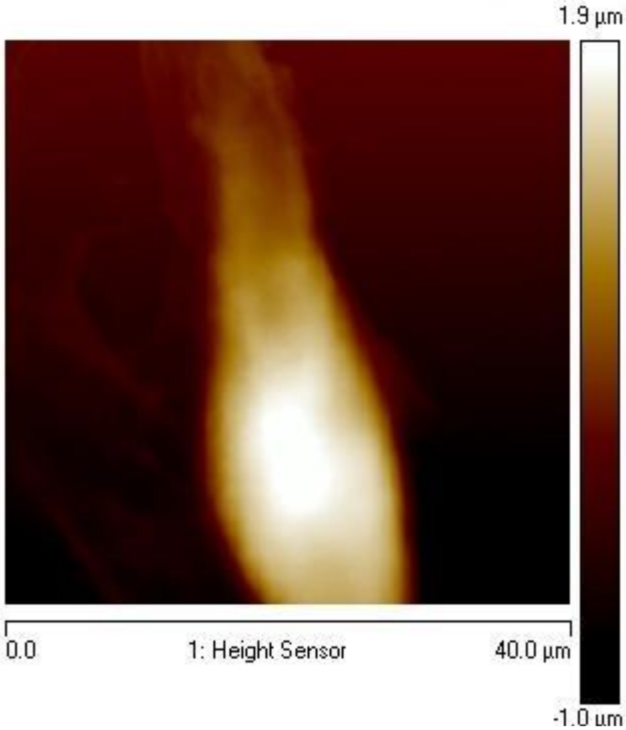


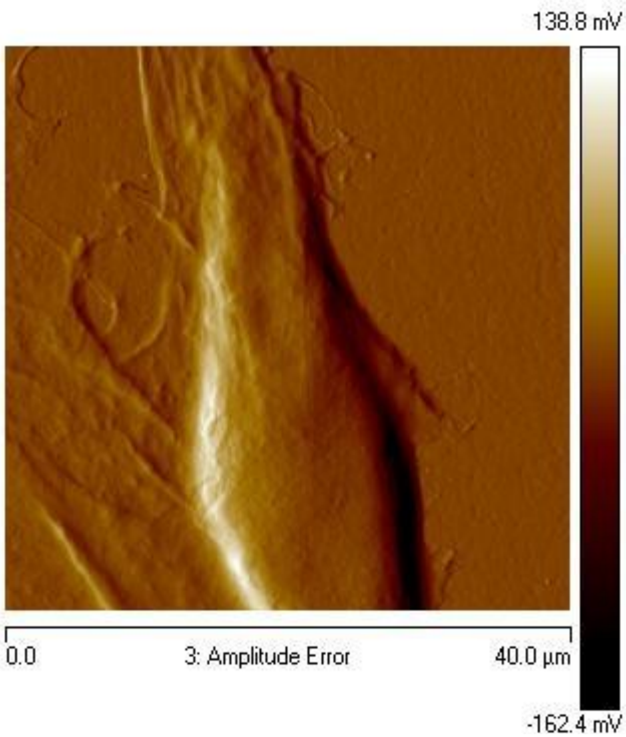
(c)

Figure 2: Untreated MC3T3 cells. (a) Optical microscope. (b),(c) AFM images of two different cells.

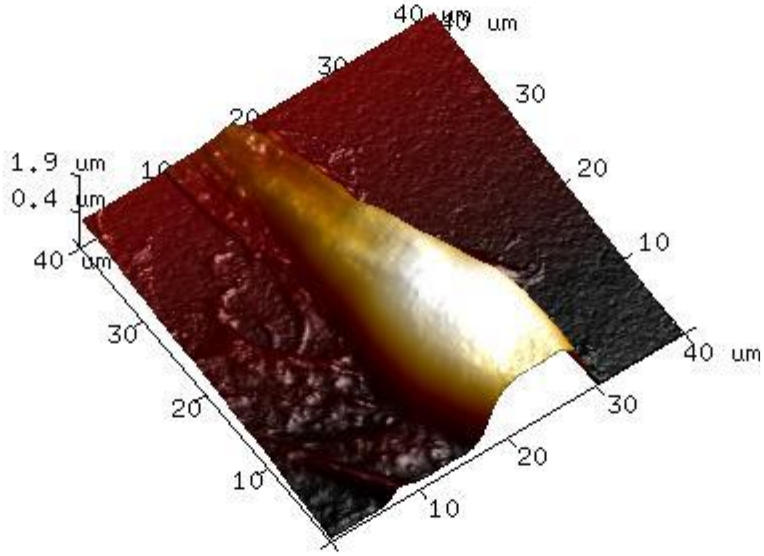
Cells in images above and below show a recognizable nucleus, average size about 30-40 micron and have an average height about 1-1,5 micron.


1.9 µm
0.0
1: Height Sensor
40.0 µm
-1.0 µm

(a)


138.8 mV
0.0
3: Amplitude Error
40.0 µm
-162.4 mV

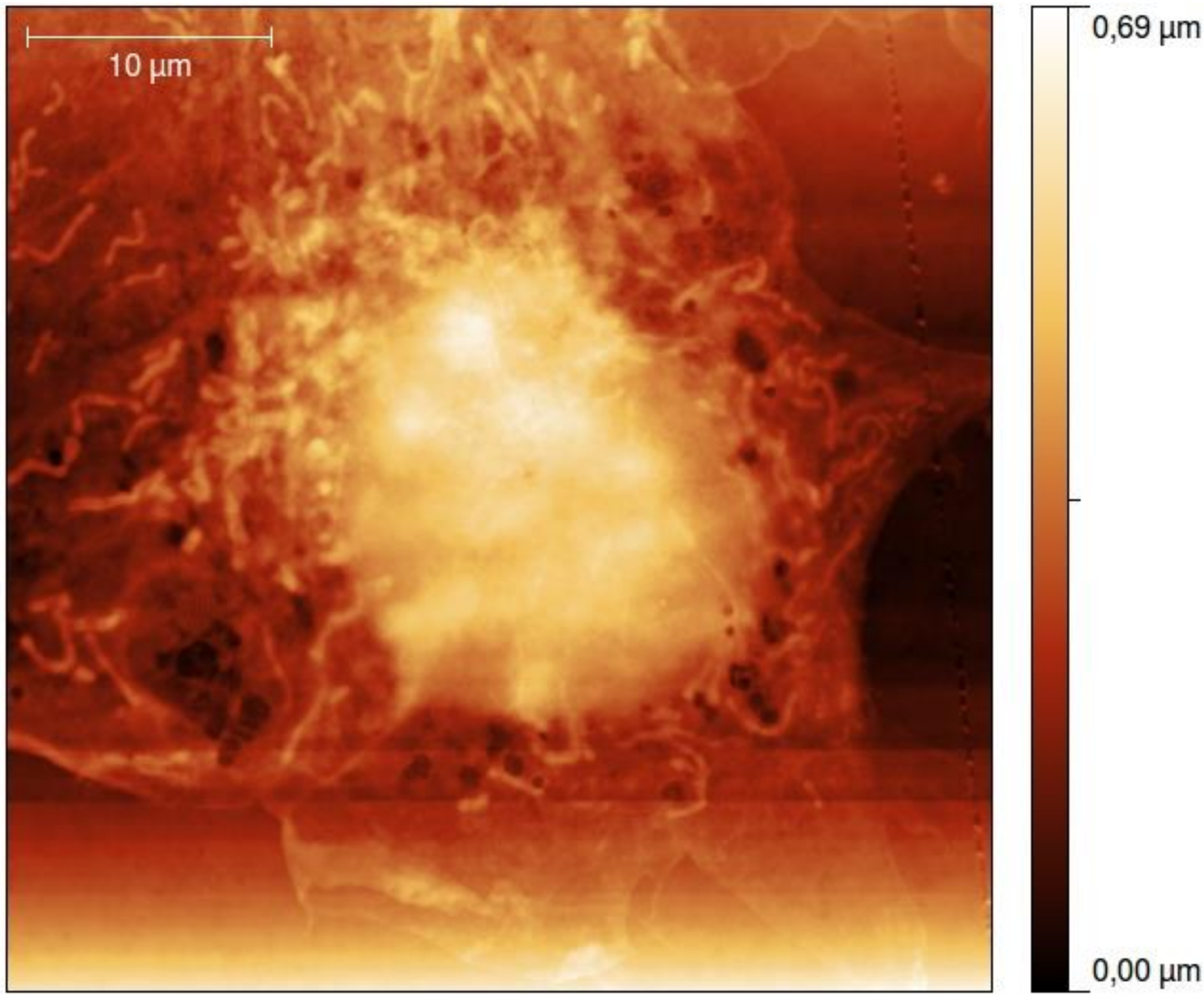
(b)



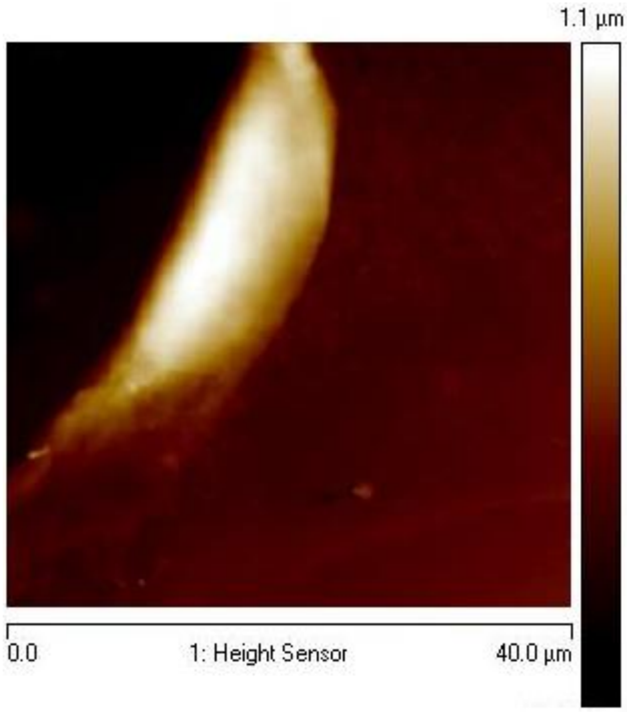
(c)

Figure 3: AFM images of an untreated MC3T3 cell. (a)Topography. (b) Error images emphasize edges and details of the surface. (c) 3D image.

Different characteristics appear in treated cells (24h in microgravity). In this latter case, in fact, the nuclei are no longer recognizable and circular. Also heights have changed and decreased to about 1mm. The images of treated cells can be seen in Fig. 4.



(a)



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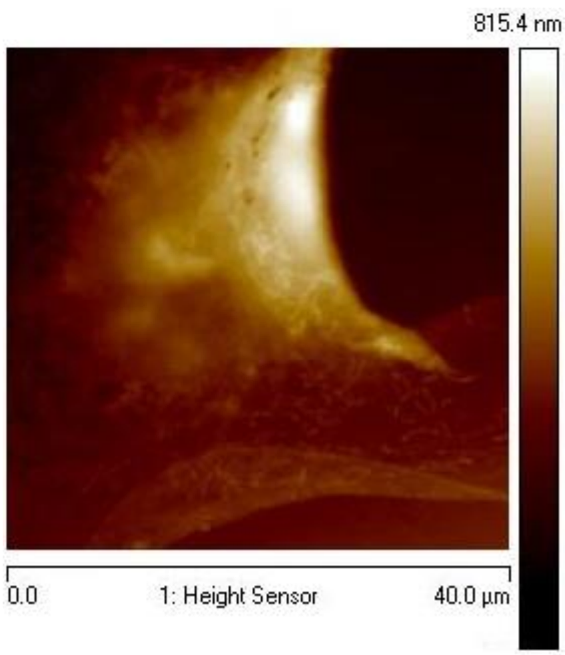
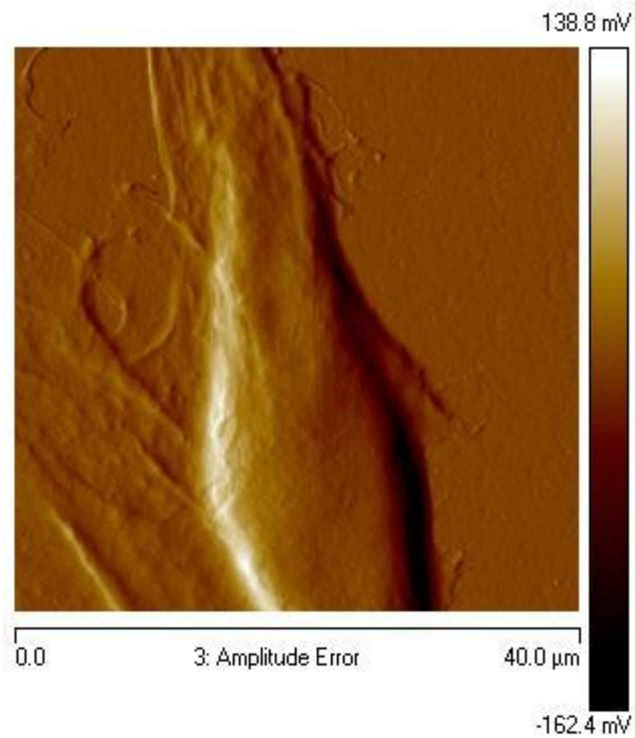


(c)

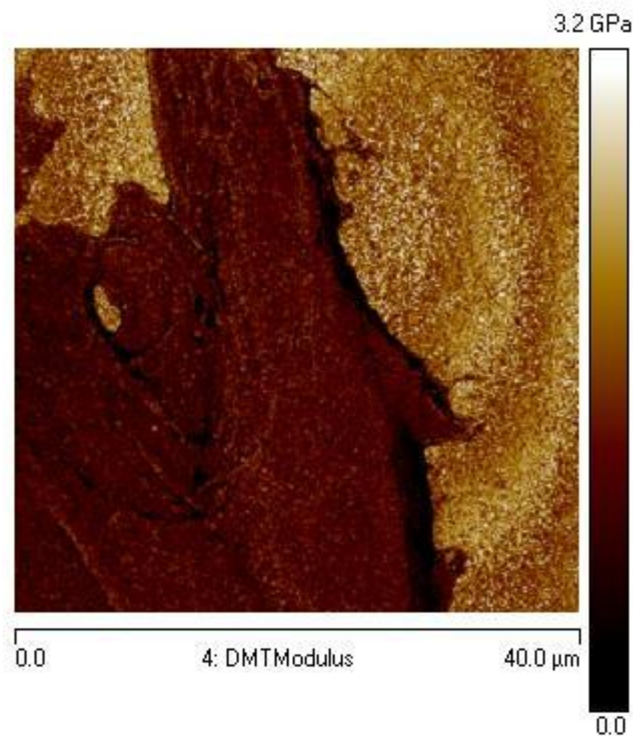
Figure 4: AFM images of treated MC3T3 cells.

Mechanical properties

Mechanical properties are characterized by the DMT modulus [3] (an elastic modulus modeled for an interaction between a tip with physical dimension and an elastic surface).
We want to compare the elastic modulus of treated and untreated cells. In the next figures we can see beside the topography the same cells with DMT modulus images.



(a)



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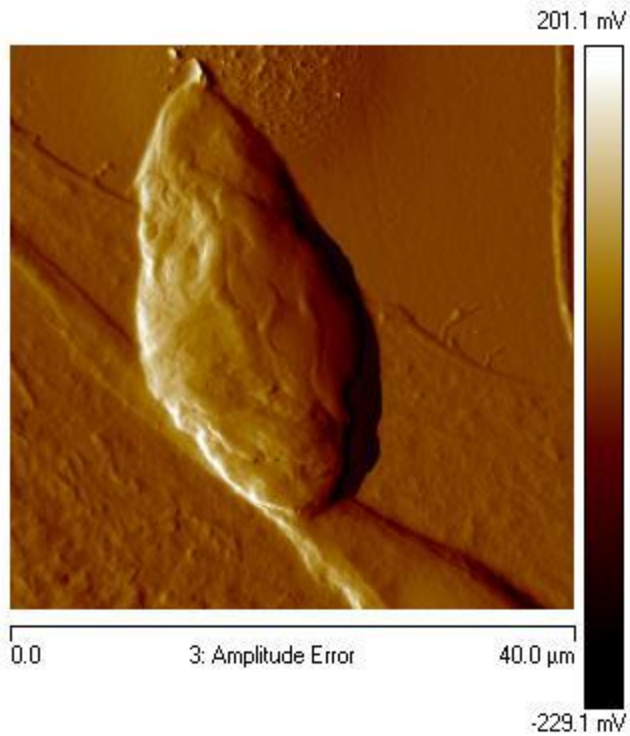
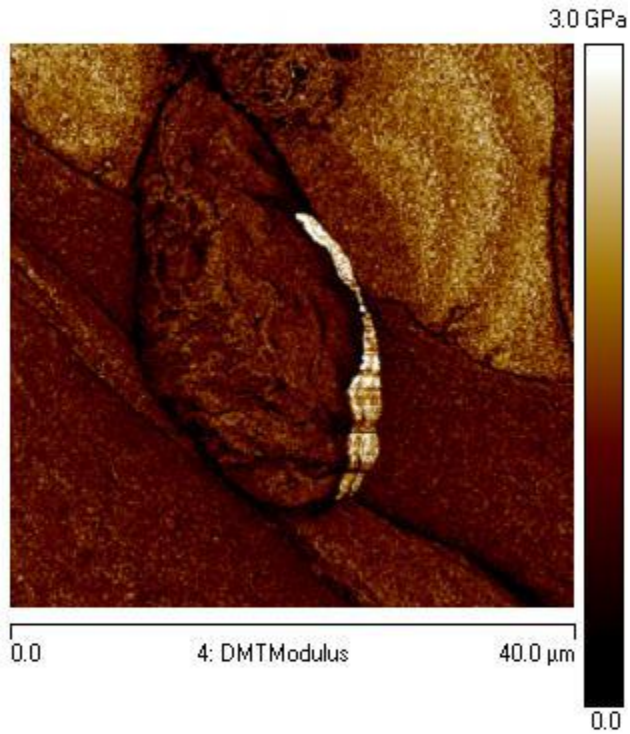


(c)



(d)

Figure 5: AFM images of untreated MC3T3 cells. (a),(c) Topography. (b),(d) Elastic (DMT) modulus.

From extraction of the profile (Fig. 6) of the images we can see that the elastic modulus of the untreated cell is significantly lower than that of the support and that in the peripheral part of the cell is lower than the elastic modulus of the central part (black circle).

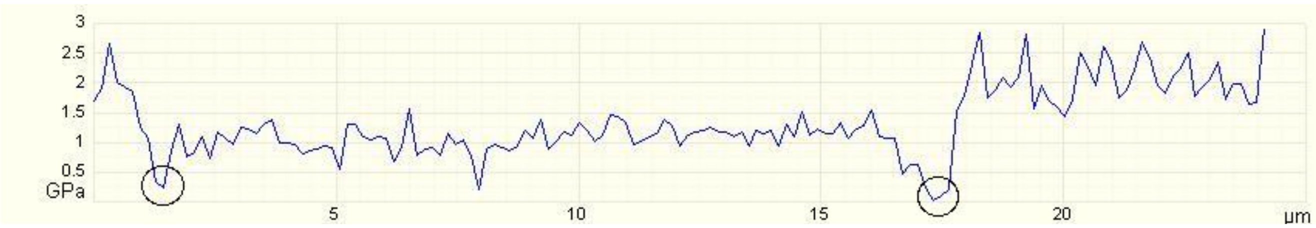
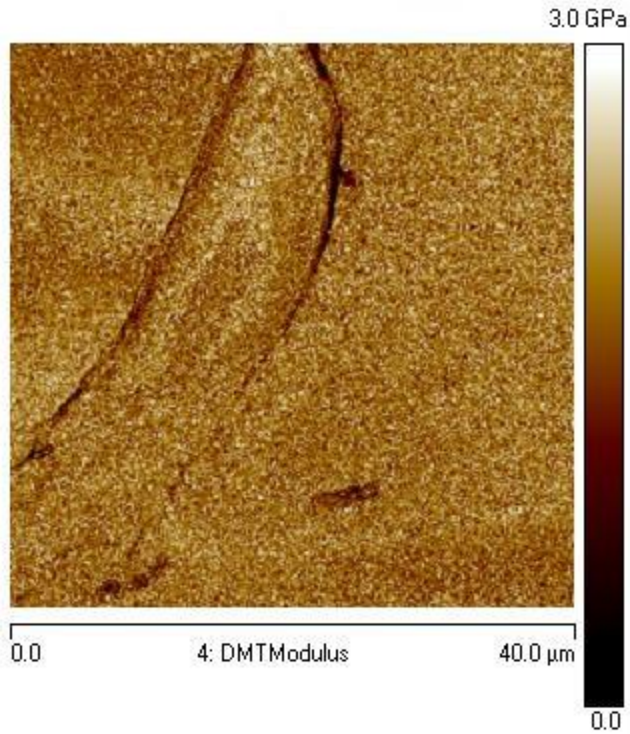
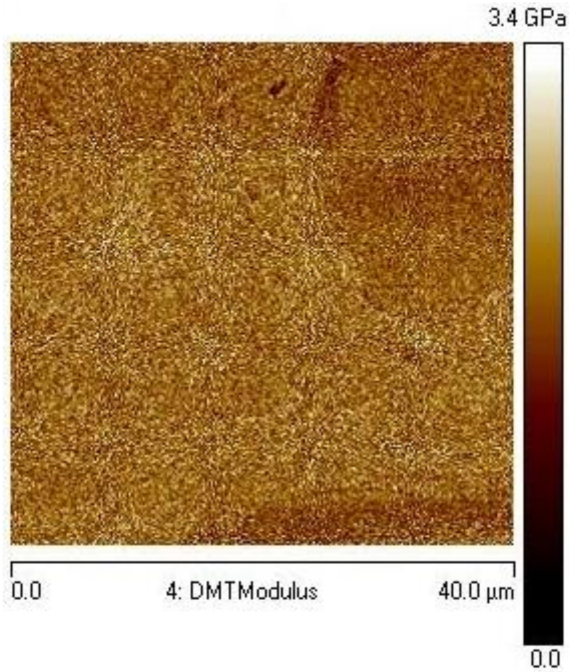


Figure 6: Profile of DMT image of an untreated MC3T3 cell.

The same analysis has been performed for the treated cells but with different results (Fig. 7). The elastic modulus of the cell is no longer recognizable than that of the support as if the cell had stiffened after treatment in microgravity.



(a)



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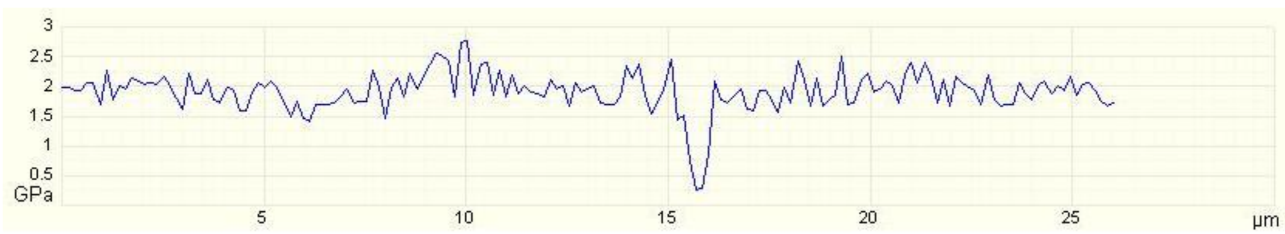

(c)

Figure 7: Elastic (DMT) modulus of treated MC3T3 cells and profile extracted from (a).

Conclusion

The preliminary analysis made here show good results in terms of comparison between treated and untreated cells. In particular we found changes in DMT modulus.
Many other tests may be performed, for example to verify different mechanical properties such as adhesion to a substrate or to verify if the effects of microgravity are reversible.
But we especially need further investigation to increase the statistics, both by increasing the number of cells and analyzing different biological samples and thus confirm the results obtained.

References

[1] "Veeco HarmoniX: User guide." Veeco Instruments Inc. 2008
[2] Sahin "Harnessing bifurcations in tapping-mode atomic force microscopy to calibrate time-varying tip-sample force measurements." Rev Sci Instrum 78 103707 (2007)
[3] Derjaguin B.V., Muller V.M., Toropov Yu.P. "Effect of contact deformations on the adhesion of

particles." J. Colloid Interface Sci 53 314 (1975)

List of Conference Talks

S. Bellucci, What Next in Condensed Matter, INFN-LNF, Frascati (Italy), Feb 27, 2015.

S. Bellucci, Research Seminar, Unical Cosenza (Italy), 10 March 2015