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

S. Bellucci (Resp. Naz.), S.Bistarelli (A.R.), A. Cataldo (A.R..), 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.

The main aim of the project is 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.

We use the Atomic Force Microscope for the morphological and mechanical characterization of cells exposed to a microgravity environment, as a way to determine the response of the living systems to adverse physical constraints, in view of using countermeasures (drugs, etc.) to counteract such effects and favor their reversibility.

Research Activity

In the previous year (2015) we obtained results after 24 hours of exposure, with the cells showing changes both in shape and in stiffness. To confirm results obtained in the first year of the project, we decided to change different parameters in cell culture. The exposure to microgravity was performed with two simulations RPM Random and RPM Partial G – carried out by a planetary rotation simulator on earth - and the object of study was replaced with H9C2 cell lines.
In RPM Random, the G* vector, the sum of gravitational and centripetal forces, changes randomly and quickly position, obtaining the microgravity.
In RPM Partial G, The G* vector changes position slower and in controlled way than RPM Random. Repeating in cyclically the sequence (recording each position during first cycle) the cells experienced microgravity slowly rotating.

H9c2 cells represent a cardiomyocyte-like cell line derived from rat embryonic hearts. These cells do not display the sarcomeric organization typical of adult cardiomyocytes reflecting their origin from late embryonic cardiomyocytes which have not yet fully differentiated. They are largely used in cell biology as model for cardio-circulatory apparatus.
The activity of these six months covered the analysis of morphology of treated and untreated cells.
AFM works in air so no special preparation of samples is required for analysis. Nevertheless, Growth and preparation of cells could leave salt residues on their surface, then fixed cells were washed three times with solution containing glucose (0.1M) and NaCl (0.1M) with ratio 2:1 to avoid any residues.

Untreated

Morphology of untreated cells are shown in Figure 1 and Figure 2.

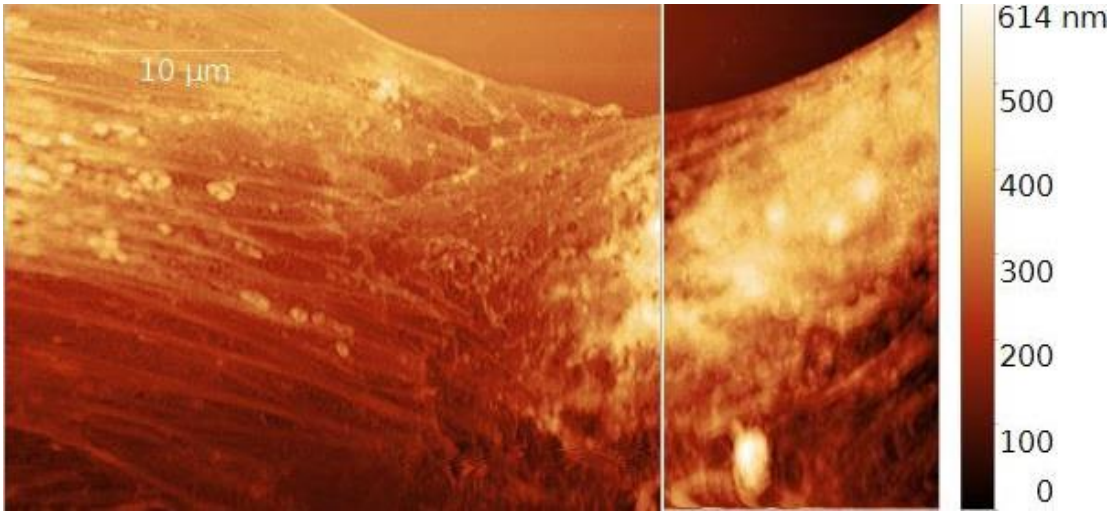

Figure 1 Untreated H9C2 cell. Merging of two images represents two overlying cells.

The average size of a cell is 40 □m exceeding the scanning size of the microscope. So to visualized a whole cell some merging of different images were performed.

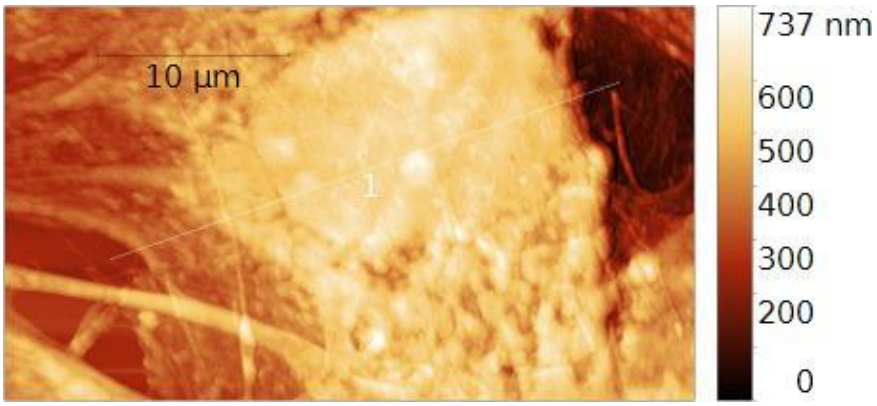

Figure 2 Untreated H9C2 cell. From this image was extracted a profile to characterize the surface of the cell.

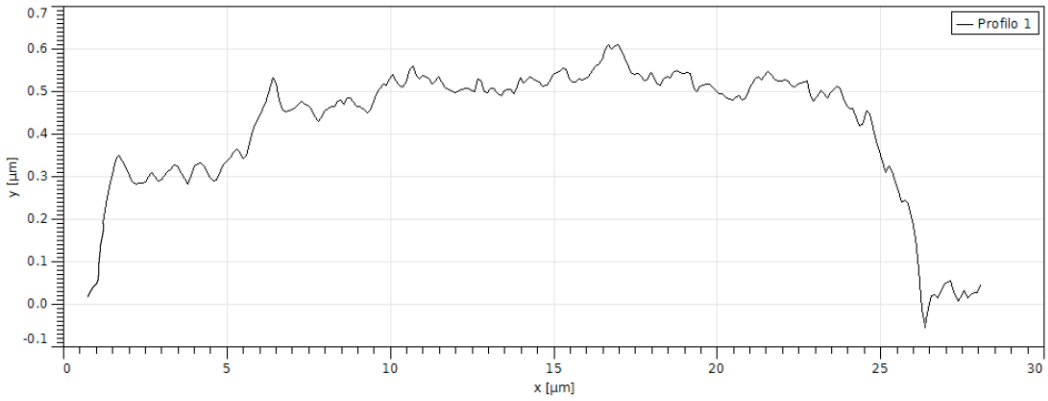

Figure 3 Profile from Figure 10.

From profile extraction we can extract some properties of the cell. The height in the area of nucleus is about 0.6 □m while in the peripheral parts tends to decrease. Another quantity analysed is the roughness on the surface of the cell. This quantity is calculated by:

$$R_{RMS} = \sqrt{\frac{1}{M \cdot N}\sum_{i=1}^{N}\sum_{j=1}^{M}\left(z_{ij} - \bar{z}\right)^2}$$

where (M·N) represents the total number of pixels in the selected area, z_{ij} is the height measured in correspondence of the ij element of the image and $\bar{z}$ the height mean value of the analyzed area.

The results for our samples is

$$R = 0.043 \pm 0.008 \mu m$$

RPM Partial g

Morphology of treated cell with RPM Random is shown in

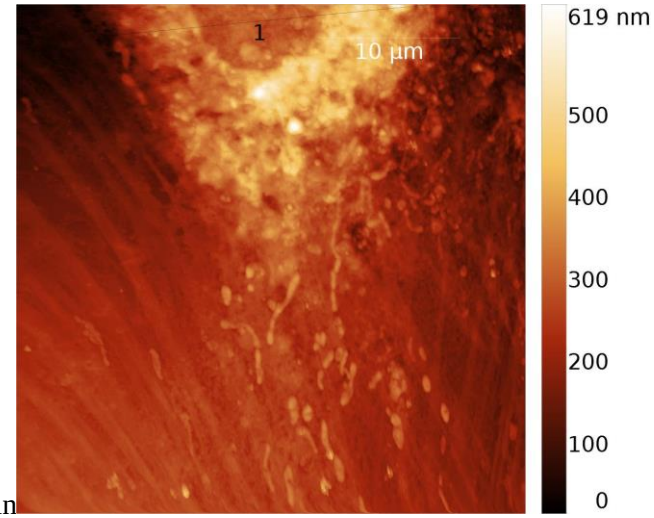

Figure 4 Treated H9C2 cell with RPM Partial g. From this image was extracted a profile to characterize the surface of the cell.

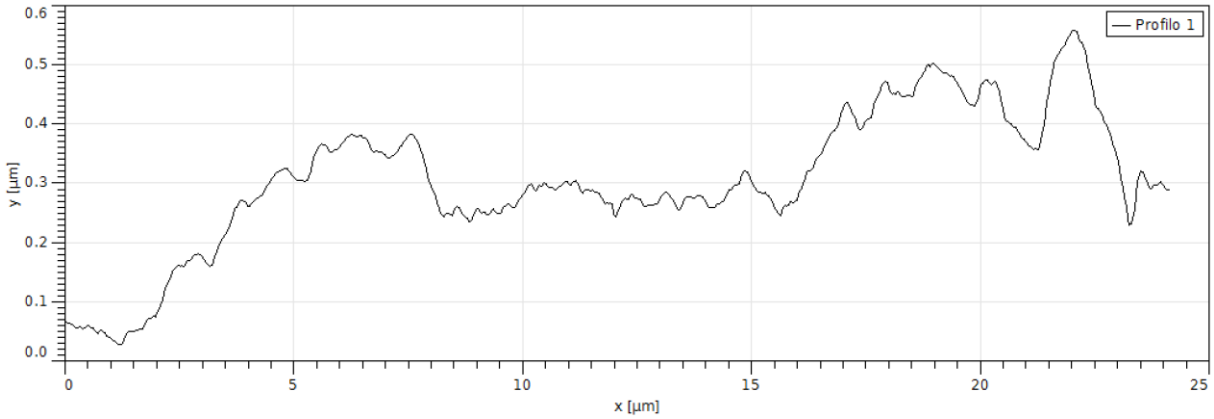

Figure 5 Profile from Figure 12.

As well visualized from the profile the surface in the area of nucleus is characterized by a lowering of the central region while maintaining the edges of the nucleus at the initial height. Also the roughness changes increasing to the value of:

$$R = 0.095 \pm 0.009 \mu m$$

RPM Random

Morphology of treated cell with RPM Random is shown in Figure 6

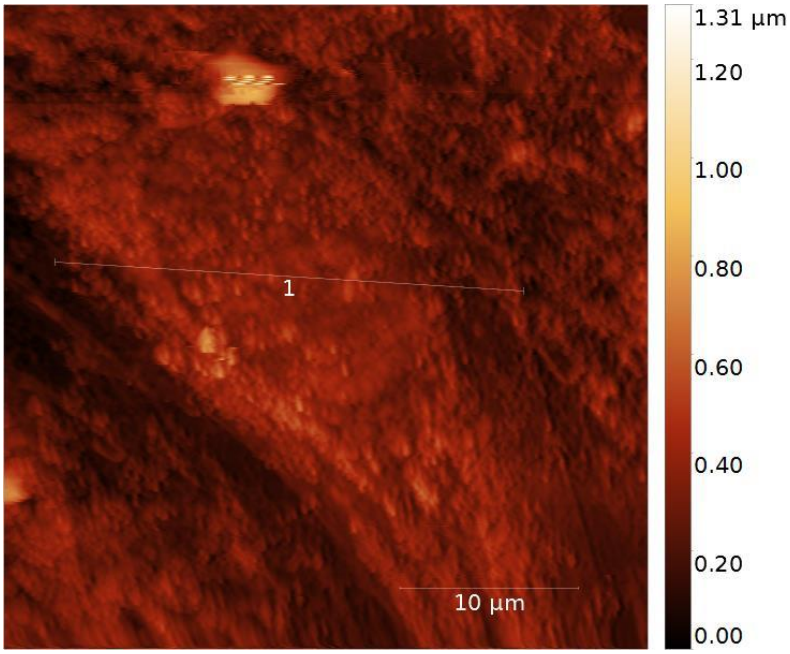

Figure 6 Treated H9C2 cell with RPM random. From this image was extracted a profile to characterize the surface of the cell.

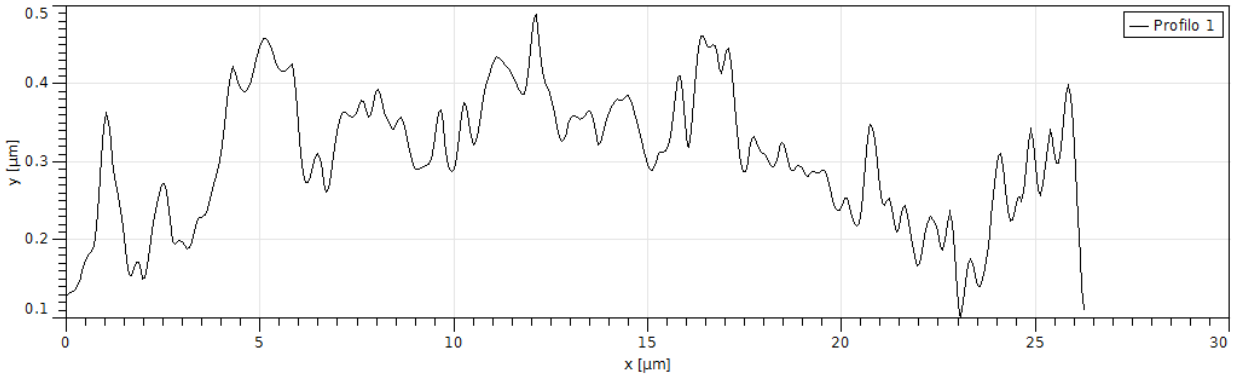

Figure 7 Profile from Figure 14.

Even in this case the treatment results in a lowering of the central region. This could be observed from the total height decreased to 0.4 micrometer. Also the roughness seems accentuated. In fact, from the calculation we find an average roughness of

$$R = 0.12 \pm 0.01 \mu m$$

Characterization with Scanning Electron Microscope

The H9c2 cells were characterized by Scanning Electron Microscopy to observe the morphology of samples.

In fig. 16 a typical micrograph of H9c2 cells is reported:

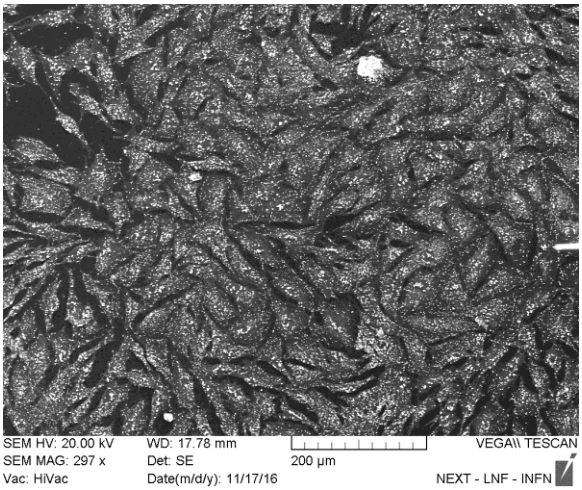

Figure 8 Micrograph of H9c2 cells completely coated by the salt

It is visible that the sample is covered by salt due to the evaporation of the solvent. We are optimizing the procedure of washing to eliminate the most part of salt.

Nevertheless, in some region it is possible to observe cells with a little coating of salt, as visible in fig. 17

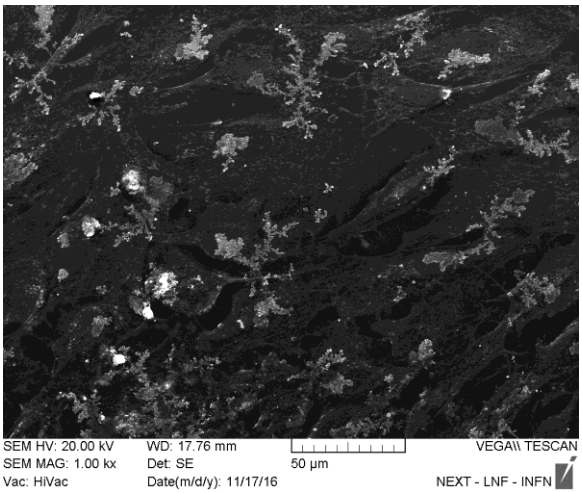

Figure 9 Micrograph of H9c2 cell partially coated

In this region the morphology of sample is visible. Furthermore, increasing magnification, shape of nucleus is visible (fig. 18).

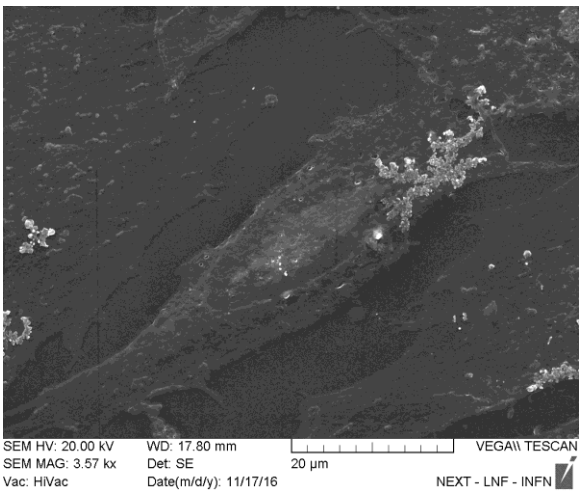

Figure 10Micrograph of H9c2 cell i n which nucleus is visible

We are going to carry out further investigation, improving the washing step to eliminate salt, to compare morphological modification due to microgravity exposure.

List of Conference Talks

S. Bellucci, Conference on Nanoscience and Nanotechnology, INFN-LNF, Frascati (Italy), 26-29 September 2016.

S. Bellucci, Research Seminar, UNIVPM Ancona (Italy), 13 May 2016

S. Bellucci, The SHAPE Project - A New Theoretical Framework of the Microgravity-Cell Interaction, 2016 EMN Summer Meeting, Cancun, June 6-8, 2016