*AUTHOR: G. Gauquelin-Koch, Life Sciences program scientist*
*CNES, 2 place Maurice Quentin, 75039 Paris, France.*

# Life Sciences in Space

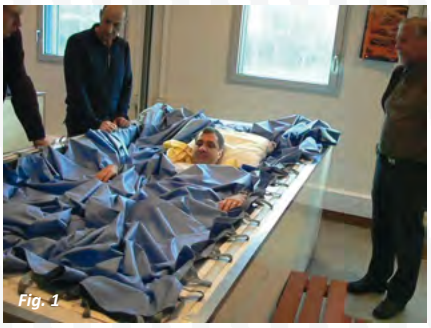
*Fig. 1*

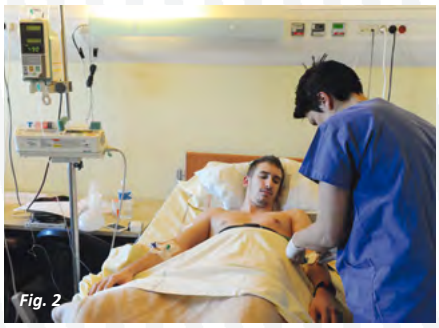
*Fig. 2*

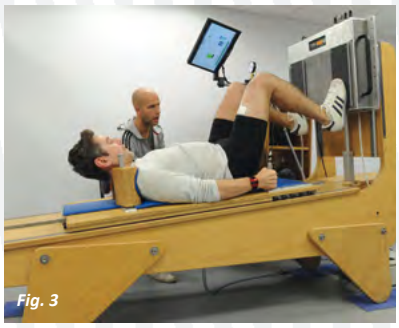
*Fig. 3*

Men have always explored, exploited, colonized and controlled their environment by expanding their field of activity. From the very first hominids, technological progress has exposed our species to extreme situations and hostile environments. But although mankind's expansion into space seems irreversible, it is still a major challenge for humans. Indeed, gravity has shaped the plant and animal world over millions of years, and we spend much of our lives resisting it.

Human bioastronautics programs have grown since the culmination of 50 years of human space flight experiment. Medical and physiological findings from these missions have demonstrated that spaceflight has a dramatic impact on almost all physiological systems including muscle atrophy, bone demineralization, cardiovascular and metabolic dysfunctions, impaired cognitive processes and reduced immunological competence, and nutrition/metabolism. These adaptive responses lead to a physiological de-conditioning in space and have the potential to affect crew health and performance both in space and upon return to Earth.

The scientific communities working on Life Sciences in the microgravity environment have access to a number of different existing space- and ground-based facilities to conduct theirs experiments: International Space Station, recoverable capsules, parabolic flights and ground simulation (bedrest, centrifuge, and immersion).

Two baths of immersion have been installed in MEDES (space clinical) in 2014. Immersion in fluid is the natural state for our cells, for our aquatic ancestors, and even for us during the 9-month prenatal period (Fig. 1). The curative properties of immersion in water have been known and exploited for centuries. Somewhat ironically, however, the current growing scientific interest in water immersion is due not to prior experiment, but rather to the development of space programs.

Weightlessness causes numerous physiological changes, which affect the musculoskeletal, cardiovascular, sensory, nervous, and other systems. Experimental opportunities during actual space flight are limited, so ground-based models are necessary (Fig. 1, 2 et 3).

These models allow the assessment of microgravity induced deconditioning effects, and reveal gravitational mechanisms in the body's physiological systems, as well as mechanisms involved in adaptation of the body to microgravity. In particular, they allow researchers to develop and test measures to counter the deleterious effects of weightlessness. Immersion is one of these models, because it creates conditions that closely resemble the gravity-free environment.

CNES has taken part in experiments on rat for 30 years with BIOCOSMOS and BION.

In 2013, it contributed to BION-M1 (Fig. 4) with three teams : cardiovascular physiology, metabolic and bone.

They aimed to study:

- regulation of blood pressure and heart rate during the whole flight by telemetry: for humans, the cycles are modified.
- numerous metabolic adaptations: it was indeed shown that both the hypokinesia and the hypodynamia induced by weightlessness affect the energy turnover and capacity to uptake circulating triglycerides and non-esterified fatty acids which, in turn, affect the whole body insulin signalling pathways. The underlying mechanisms have not been properly studied and several hypotheses were made. They involved specific regulatory proteins in relation to energy and lipid turnover and the accumulation of lipid intermediaries that may interfere with both insulin and mitochondrial functions. Differential proteomics and metabolomics approaches have been suggested in order to determine the metabolic network at hand during the exposure of mice to actual microgravity. Those data will be compared to the mechanisms involved in humans during bed rest and for which we are currently analysing soleus and vastus lateralis biopsies. Over the long-term, those results will help to delineate new levers, which can be used to develop a new generation of countermeasures.


Fig. 4

- knowledge of trabecular bone alterations and concomitant bone cellular changes. In brief, a bone loss with less trabeculae – the remaining ones being thinner – were reported. Bone formation was regularly reported to have decreased while resorption activity was not always impaired. These cellular alterations might be different from those which were reported, based on serum or urine markers, in human cosmonauts. Rodents are still growing and the bone loss seen is also the consequence of altered longitudinal and periosteal expansion.

The new integrative physiology's approach is necessary for bioastronautics research. This integrative approach is obviously enhanced by recent developments in molecular biology and new analytical technologies such as the "omics".

Bioastronautics research has applications in Earth medicine particularly on the physiopathology of metabolism: nutritional questions related to bioastronautics research are very relevant to multiple ground-based related health issues. Potential spin-offs are of a great clinical importance (metabolic syndrome, insulin resistance, dyslipidemia, diabetes, etc.).

The musculoskeletal system is essential for working, locomotion and posture. Maintaining its integrity is essential to mission completion as well as astronaut health during and after the mission. Without countermeasures, all components of the musculoskeletal system adapt themselves to the microgravity environment, and by that way can create damage to tissue structure and function. Bone mass and strength are lost, muscle mass is also lost and morphology is altered, with a loss of muscle strength and endurance. The articles presented by Dr Chopard and Guigandon in this report will review our knowledge of this problem.


Fig. 5

*[Fig. 1]*
*Bath of dry immersion.*
*© Claude Gharib*

*[Fig. 2 et 3]*
*Bedrest campaign 2012-2013 at MEDES.*
*© CNES/Emmanuel GRIMAULT, 2012*

*[Fig. 4]*
*Block BIOS containing five cages for the MTB project. The MTB projects (Mice Telemetry in BION) is included in the Russian scientific program BION-M1 and focuses on a dynamic study of cardiovascular parameters of mice, during each phase of flight.*
*© CNES, 2010*

*[Fig. 5]*
*Second phase of the bedrest study conducted at the Toulouse space clinic. Measurement of basal metabolism via indirect calorimetry.*
*© CNES/Emmanuel GRIMAULT, 2013*

Laboratory contribution

AUTHOR: A. Chopard[1]

[1] DMM, UMR 866, Université Montpellier 1, 2 place Viala, 34060 Montpellier, France.

# Life Sciences in Space

## *Using transcriptomic to define muscle deconditioning with microgravity and evaluate countermeasures*

## Utiliser la transcriptomique pour définir/prévenir le déconditionnement musculaire en microgravité

➔ **Abstract:** Microgravity has a dramatic impact on human physiology, illustrated in particular, with skeletal muscle impairment. A thorough understanding of the mechanisms leading to loss of muscle mass and structural disorders is necessary to define efficient spaceflight countermeasures. Our objective is to use transcriptomics, a commonly used large-scale approach. This would allow us to make timely and more accurate predictions on the nature of the networks that vary in atrophying muscle and will strongly contribute to designing optimal countermeasures.

➔ **Résumé :** L'environnement en microgravité entraîne une fragilisation et un déconditionnement musculaire. Notre objectif est, à l'aide de techniques de transcriptomique, d'élaborer une large base de données et d'étudier les effets de la microgravité, réelle ou simulée, sur le transcriptome des muscles striés squelettiques. Ces travaux contribuent à la définition du déconditionnement musculaire et particulièrement à l'évaluation et l'optimisation des contremesures.

Current missions on the International Space Station (ISS) and future Moon or Mars exploration involve long-term manned missions. In this context, several studies have previously demonstrated the negative impact of microgravity on human physiology and, especially, the detrimental effects on the structure and function of the musculoskeletal apparatus [1].

From the last four decades of intense space-related medical research, it is clear that limb skeletal muscle is particularly sensitive to microgravity-induced deterioration in both structure and function. Muscle deconditioning is mainly characterized by a loss of muscle mass, decreased fiber cross-sectional area, reduced force, changes in phenotype, increased fatigability, insulin resistance, and fat infiltration. These changes translate into severe mobility impairment thereby reducing considerably the overall work capacity [2].

Transcriptomics have for many years contributed to increasing our knowledge of the events involved in skeletal muscle atrophy by providing detailed profiling information and accurate descriptors [3]. Together with powerful bioinformatics software, transcriptomics appears as a commonly used large-scale approach to study atrophy.

One of the objectives of such studies is to develop relevant biomarkers and molecular signatures of the atrophying and remodeling processes.

Moreover, these systematic and powerful tools also contribute to the identification of additional signalling pathways that may be involved in mediating the atrophic response. This unique and innovative approach allows us to make timely and more accurate predictions on the nature of the networks that vary in atrophying muscle during simulated microgravity and strongly contribute to designing optimal countermeasures [3].

During ESA MEP bed rest study (Cologne, DLR) we investigated the effects of 21 days of head-down-tilt bed rest in ten healthy male test subjects.

The study was conducted in a classical cross-over design [4]. The main hypothesis of MEP study was that supplementing high protein intake (1.2 g/kg body weight/d plus 0.6 g/kg body weight/d whey protein) with alkaline salts (90 mmol potassium bicarbonate/d) would maintain lean body mass during bed rest without increasing bone resorption.

Pre- and post-bed rest soleus biopsies from subjects (control and nutrition group) have been frozen in liquid nitrogen and stored at -80 °C until analysis. Classic and validated protocols have been used for RNA isolation and analysis. 200 ng of total RNA samples have been labeled with Cy3 dye using the low RNA input QuickAmp kit (Agilent) and labeled cRNA probe have been hybridized on SurePrint G3 Human Gene Expression 8x60K v2 Agilent microarrays. Microarray data analysis was performed using functions of the limma package available from Bioconductor (http://www.bioconductor.org). Data were normalized using the quantile algorithm. A linear model approach was used to estimate log fold-changes, and compute moderated t-statistics and p-values for each comparison of interest. A p-value below 0.05 after Benjamini-Hochberg adjustment was considered significant. Data from expression microarrays were analyzed for enrichment in biological terms (Gene Ontology molecular function and canonical pathways) and biological networks were built using Ingenuity Pathway Analysis software, as shown by an example on Fig. 2C (http://www.ingenuity.com/).

Our preliminary results show a total of 1 170 differentially expressed genes, selected in control and nutrition group. We identified 969 mRNAs whose expression was modified in

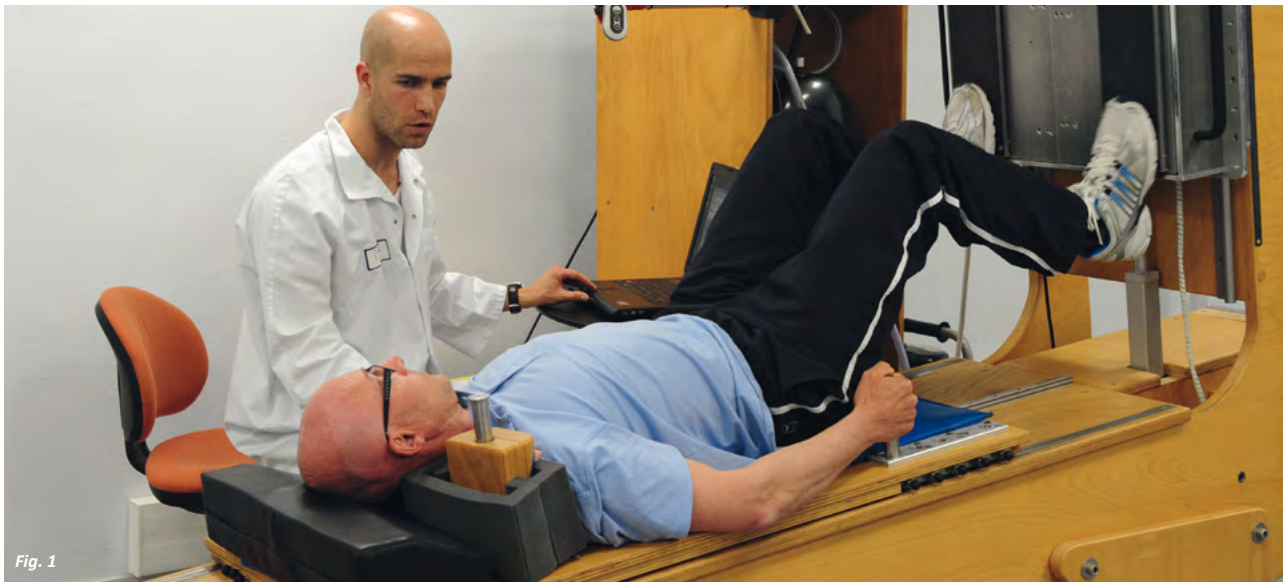

Fig. 1

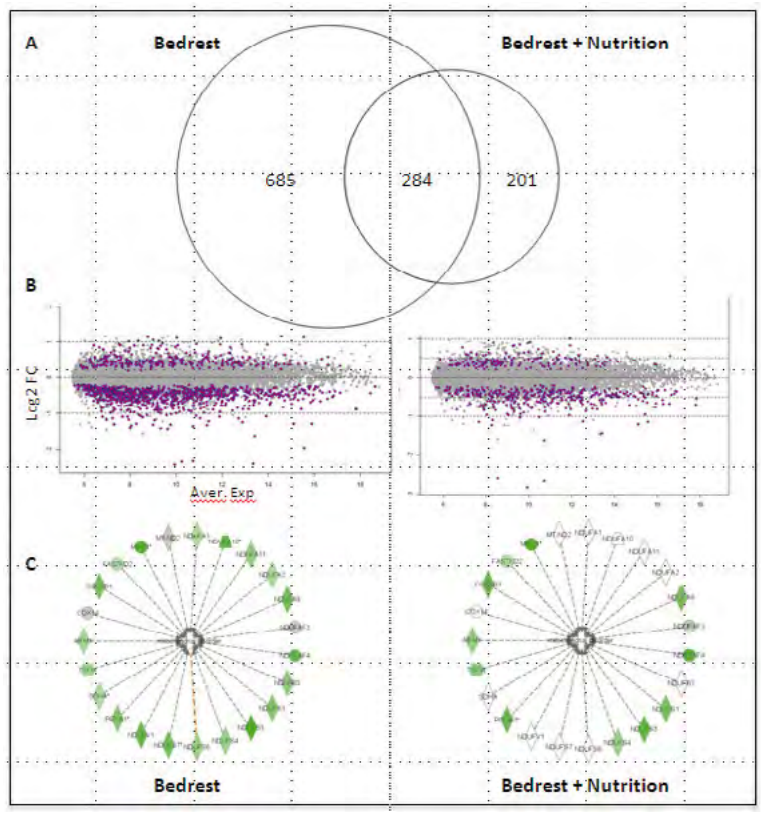


Fig. 2

*[Fig. 1]*
*Second phase of the bedrest study conducted at the Toulouse space clinic.*
*© CNES/Emmanuel GRIMAULT, 2013*

*[Fig. 2]*
*Preliminary results from skeletal muscle microarray analysis.*
*A: Venn diagram showing the number of differential genes in both groups after bed rest.*
*B: MA-plots of microarray data in both groups.*
*C: Example of functional networks (mitochondrial functions) obtained with Ingenuity Pathway Analysis software (green colour: downregulated genes).*
*© Agnès Chopard*

soleus during bedrest only and 485 during bedrest + nutrition (Fig. 2). Nutrition countermeasure demonstrated compensatory effects, decreasing the number of differentially expressed mRNAs by 50%.

These results will allow us to build and complete a large database of the changes occurring during bed rest and those triggered by the different countermeasures. Together, our transcriptomics approach will contribute to obtaining a comprehensive overview of the signaling pathways and functional networks involved in skeletal muscle remodeling during simulated microgravity and strongly contribute to designing optimal countermeasures.

## REFERENCES

[1] Fitts, R.H., Riley, D.R., and Widrick, J.J. (2001), Functional and structural adaptations of skeletal muscle to microgravity, *J Exp Biol*, **204**, 3201-3208.

[2] Chopard, A., Hillock, S., and Jasmin, B.J. (2009b), Molecular Events and Signalling Pathways Involved in Skeletal Muscle Disuse-induced Atrophy and the Impact of Countermeasures, *J Cell Mol Med*, **13**, 3032-3050.

[3] Chopard, A., Lecunff, M., Danger, R., Lamirault, G., Bihouee, A., Teusan, R., Jasmin, B.J., Marini, J.F., and Leger, J.J. (2009a), Large-scale mRNA analysis of female skeletal muscles during 60 days of bed rest with and without exercise or dietary protein supplementation as countermeasures, *Physiol Genomics* **38**, 291-302.

[4] Buehlmeier, J., Mulder, E., Noppe, A., Frings-Meuthen, P., Angerer, O., Rudwill, F., Biolo, G., Smith, S.M., Blanc, S., Heer, M. (2014), A combination of whey protein and potassium bicarbonate supplements during head-down-tilt bed rest: presentation of a multidisciplinary randomized controlled trial (MEPstudy), *Acta Astronautica*, **95**, 82-91.

Laboratory contribution

*AUTHOR: A. Guignandon[1]*

*[1] LBTO, U1059, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne Cedex 2, France.*

# Life Sciences in Space

## *Bone cells in microgravity-related conditions, a fight-or-flight response?*

## Les cellules ostéoformatrices en microgravité, une réponse au stress de type « combattre ou fuir » ?

➔ **Abstract:** Bone cells exposed to real microgravity display alterations of their cytoskeleton and focal adhesions (FA), two major mechanosensitive structures. Cytoskeleton (tension) is associated with FA stabilization (Fight) and its depolymerization with FA dispersion and migration (Flight). Osteoblast exposed to microgravity related conditions presented a Flight behavior as opposed to a Fight behavior under 1*g* conditions. Spaceflight effects could be related to excessive FA turnover, events that could be studied using live imaging.

➔ **Résumé :** Les ostéoblastes exposés à des conditions de microgravité présentent des altérations de leur cytosquelette et de leur adhérence focale (FA), deux structures mécanosensibles. La tension du cytoquelette est associée à la stabilisation des FA (Adhérence/« Combat ») et sa déstabilisation en vol est associée à la migration (« Fuite »). Nos ostéoblastes présentent donc un comportement de fuite en microgravité correspondant à leur incapacité à stabiliser des FA. Ce comportement cellulaire s'explique surement par un fort renouvellement des FA, ce qui pourrait être vérifié en imagerie dynamique.

Cell shape changes and cytoskeletal alterations have been reported by several investigators under microgravity conditions in both adherent and nonadherent cells [1]. Adhesion plaques (FA) that link the intracellular cytoskeleton to the extracellular matrix have a recognized role in mechanotransduction. Our studies in real microgravity provide quantitative data on cell adhesion after 48 to 69 h of microgravity exposure. We collected different types of cell adhesion information on two types of cells. The first type was specifically related to integrin-mediated adhesion and was based on the detection of vinculin and phosphotyrosine at focal adhesion sites by immunostaining.

The second type was not specific and represented physical contact zones at cell/substratum interface as imaged by TRIFM. The image analysis software provided 18 morphometric features describing cellular area, shape, and proportions of vinculin (and phosphotyrosine) spots as well as six topographic features describing the distribution of vinculin.

The parameters mostly affected, for both osteoblasts-like cells, by space exposure were related to FA, suggesting that integrin-dependent adhesion was an effector of microgravity-related changes. The kinetics of vinculin-related changes included a decrease in the mean area of vinculin spots, followed by a more peripheral relocation of spots (Fig. 1).

The mechanical environment is known to be crucial for the maintenance of cell integrity, because the cytoskeleton-generated tension forces that equilibrate external tension are mainly provided by cell-matrix and cell-cell interactions, pressures, and fluid shear stresses. This concept, called tensegrity [2], has led us to the assumption that the alteration of external forces (reduced *g* level, shear stress, and pressure) leads to unbalanced cytoskeletal tension at the beginning of the flight and that longer exposures will lead to a reduction of cytoskeleton generated tensions.

In regard to this theory, our results showing disappearance of stress fibers as well as disassembly of focal complexes fit well with the assumption of a reduction in membrane contractility when cells are exposed to microgravity. This assumption also fits with the concept known in physiology that cells unable to "fight" (adhere and maintain cell tension) are engaged in migration (dispersion of FA, loss of stress fibers (*i.e.* cell tension)) and adapt to this stressful situation by adoption of a "flight" type response. It has been shown that turnover of focal adhesions was found to be negatively correlated to cell tension [3].

LBTO's studies confirm that exposure of osteoblastic cells to microgravity impairs their cytoskeleton stability, and reduces cellular tension as well as focal adhesion formation or stability [4] leading to reduced fibronectin deposition in MG63 [6]. Harrison's group recently reported similar results and also that zyzin positive contacts were almost absent from contacts established during flight, in osteoblasts thus confirming that focal adhesions in microgravity were less mature than those formed under 1*g* conditions [1].

A general picture clearly emerges: in microgravity-related conditions, osteoblasts will form focal complexes (small, clustered structures, located at the cell edge) that cannot be stabilized into mature focal contacts by internal tension. FA formed in microgravity may present an important turnover due to the reduced cell tension, which would explain their inability to mature into focal/fibrillar ones and to support fibrillogenesis/matrix deposition. Furthermore, matrix degradation by MMP, known to be up-regulated in bone exposed to microgravity, may also account for the inability of osteoblasts to adhere

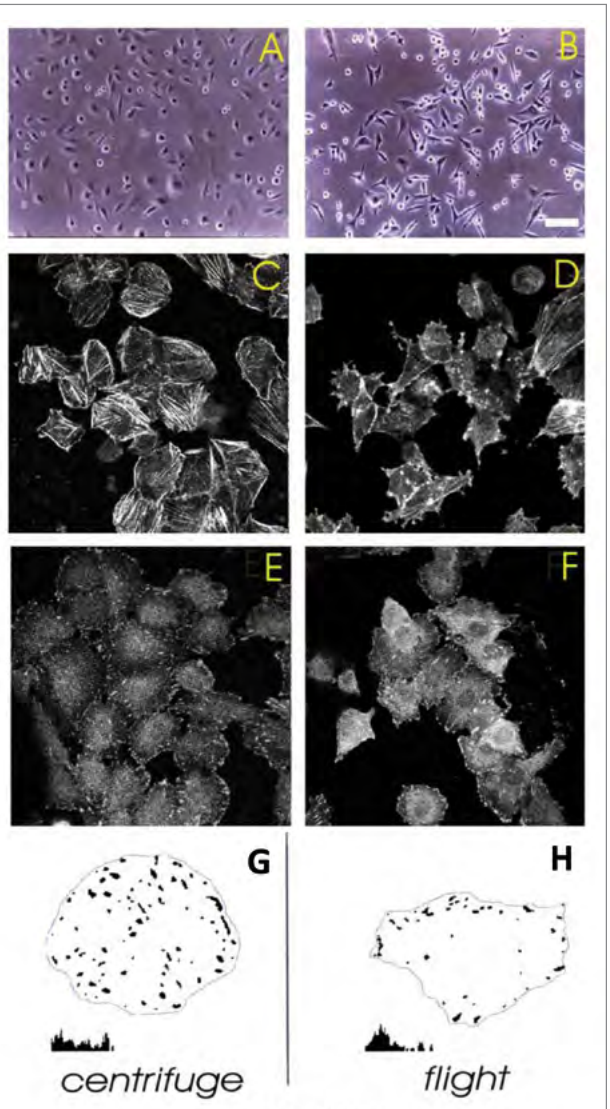


Fig. 1

*[Fig. 1]*
*Observation of ROS172.8 cells after spaceflight exposure.*
*(ACEG): Centrifuge inflight controls and (BDFH) flight culture:*
*(AB): Phase contrast microscopy.*
*Note that after 4 days a large proportion of flown cells are retracted or round compared with the well-spread morphology of centrifuge.*
*(CD): Vinculin staining. FA obtained in confocal microscopy analysis of the 2-day centrifuged group of cells.*
*(EF): f-actin staining illustrating cytoskeleton integrity.*
*(GH): Vinculin representative pattern in microgravity-exposed cells indicating an important redistribution of vinculin to cell edges in flight cells. Bars: 15 µm.*
*© From [4]*

and differentiate in microgravity [6]. There is now a clear need for the community of live cell imaging in microgravity-related conditions in order to establish the dynamic of cytoskeletal proteins and FA to suggest more advanced explanations of the observed "Fight or Flight" response.

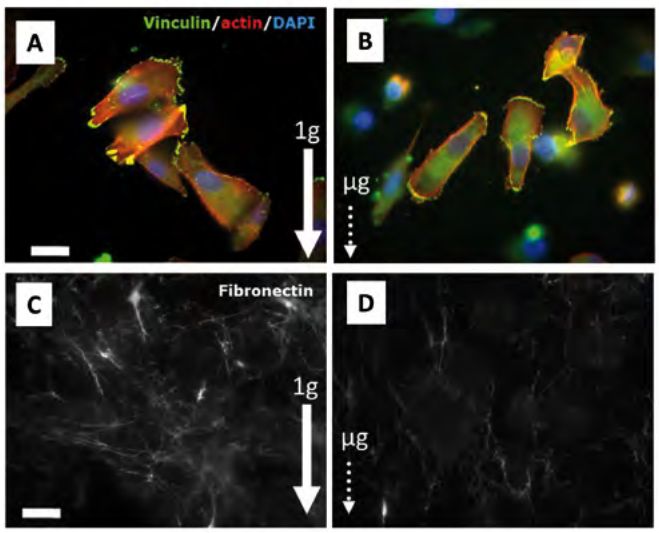


Fig. 2

*[Fig. 2]*
*Morphological description of MG-63 cells after exposure to microgravity. (AB) The cells were processed for detection: of focal adhesions by using vinculin antibody; of stress fibers by using rhodamine phalloidin staining. DAPI was lastly used for nucleus revelation.*
*(A): 1g conditions (ground controls);*
*(B): µg-related conditions.*
*(CD) Images of fibronectin deposited by MG-63 cells after exposure to microgravity.*
*(C): 1g conditions (ground controls);*
*(D): µg-related conditions. Cells were processed for detection of fibronectin by using a specific antibody*
*Bar: 10 µm.*
*© From [5]*

## REFERENCES

[1] Nabavi, N. *et al.* (2011), Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion, *Bone*, **49**, 965-974.

[2] Ingber, D.E. (2006), Cellular mechanotransduction: putting all the pieces together again, *FASEB J.*, **7**, 811-827.

[3] Xiang-Dong, R. *et al.* (2000), Focal adhesion kinase suppresses Rho activity to promote focal adhesion turnover, *J. Cell Sci.*, **113**, 3673–3678.

[4] Guignandon, A. *et al.* (2001), Cell cycling determines integrin-mediated adhesion in osteoblastic ROS 17/2.8 cells exposed to space-related conditions, *FASEB J.*, **11**, 2036-2044.

[5] Guignandon, A. *et al.* (2014), Rac1 GTPase silencing counteracts microgravity-induced effects on osteoblastic cells, *FASEB J. in revision.*

[6] Carmeliet, G., *et al.* (1997), Microgravity reduces the differentiation of human osteoblastic MG-63 cells, *J. Bone Miner. Res.*, **5**, 786–794.