Osteoclast gene expression and resorption during spaceflight

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Abstract

During space exploration astronauts are exposed to the microgravity environment, which has an immediate impact on many biological systems. Osteoporosis-like bone mass loss is one of the most significant effects of microgravity, with reduction ranging around at least 1-2% per month in flight. An uncoupling of bone remodeling could be responsible for this process. Data obtained from astronauts showed up to 38% decrease in bone formation serum markers, while resorption markers were increased to 78%. We investigated the biological role of osteoclasts in microgravity-induced bone loss with experiments performed during FOTON M3 ESA Mission in September 2007. We studied osteoclast differentiation from mouse-derived isolated monocyte precursors and bone resorption by mature osteoclasts, and found that microgravity directly stimulates osteoclastogenesis and increases bone resorption. These evidences indicate osteoclasts as direct target of mechanical forces and further address future studies to the understanding of the cellular and molecular mechanisms of osteoclast behavior in microgravity.

Key Words: osteoclast, bone, osteoporosis, bone resorption

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Prolonged microgravity experienced during spaceflight missions seems to affect many physiological systems. One of the most significant effects is an osteoporosis-like loss of bone mass. The rate and extent of this process is considerable, with a decrement of 1-2% total bone per month in flight [4]. This reduction may be caused by an increase in bone resorption and decrease in bone formation leading to an uncoupling of bone remodeling. Human data obtained from long spaceflights (from 14 days to six months) showed a 38% decrease in bone formation serum markers and up to 78% increase of bone resorption markers during flight [2, 6, 8].

The current space program requires that astronauts will be involved in longer missions. The implementation of a new international space station and the human exploration of Mars are being planned by international space agencies, but the biomedical problems associated with long duration space flight [3] must be solved before these missions can take place, otherwise the prolonged exposure to the space environment may result in significant health consequences. This bone demineralization, with its resultant hypercalcaemia and hypercalciuria, would leave crews at substantially increased risk of pathological fractures and renal calculus formation.

It is possible that both the bone resorption and formation are affected from microgravity. Experiments on pre-osteoblasts cultured in microgravity simulated conditions demonstrated the down regulation of differentiation related genes as ALP and RUNX2 [9, 13].

Microgravity is also a perfect tool for understanding the role of mechanical forces on single cell population. While several reports on the role of mechanical forces on the osteoblastic lineage are already available [1,6,7,10], it is not known if osteoclasts and/or their precursors are a direct target of these forces, or if the indubitable effects observed in vivo are only osteoblast mediated. Ex-vivo cultures of marrow osteoclast precursors, obtained from rats after a tail-suspension period, showed an increase in osteoclastogenesis, but did not elucidate if this result was direct or osteoblast mediated [5].

Recent data showed an increase in bone resorption activity in osteoclast supplemented with conditioned media from osteoblast grown in simulated microgravity conditions [11].

In this paper we investigated the biological role of osteoclasts in microgravity-induced bone loss during LIFE FOTON M3 Mission in September 2007. We studied osteoclast differentiation from mouse isolated

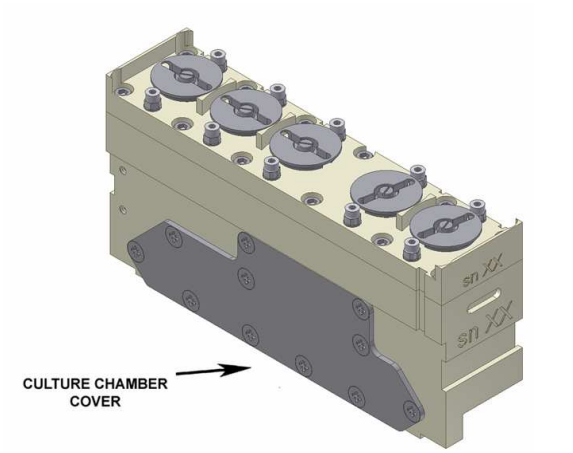

Fig. 1 *The PITS bioreactor is composed by a brick of biological compatible plastic where five cylinders, for the chemicals, a culture chamber and connecting channels are machined. The Figure shows the STR cell, without the electronic board, showing the culture chamber position.*

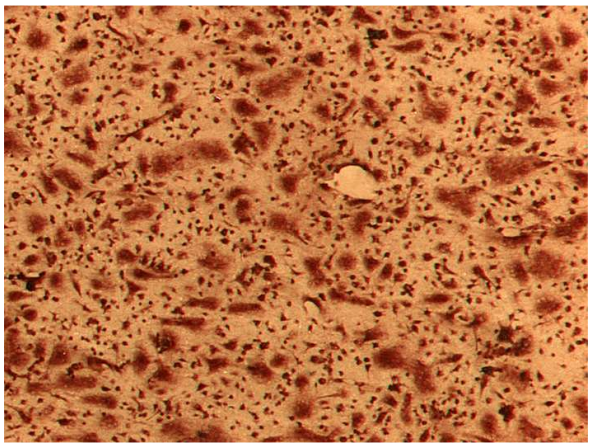

Fig. 2 *Bone specimens were controlled during the osteoclast precursor differentiation. After 4 days of culture in the presence of 10ng/ml MCSF and 30ng/ml RANK-L, the bone slices appeared colonized by cells. Mature osteoclast are visible on the bone surface by phase contrasts.*

monocyte precursors and bone resorption by mature osteoclasts, in three different experiments, OSPACE - OSTEO, OCLAST and PITS with the support of the Italian Space Agency (ASI). Here we report the preliminary data obtained in PITS experiment. Our results show that microgravity directly stimulates osteoclasts and increases bone resorption in the absence of osteoblasts, indicating osteoclasts and their precursors as direct target of mechanical forces and further address future studies in order to understand the

The experiment is automatically run by the internal microcontroller executing the preloaded timeline; the activation times of the five pistons could be changed by means of the EGSE. Figure 1 shows the STR cell, without the electronic board, showing the culture chamber position. The prototypes have been developed by Kaiser Italia, Livorno, Italy.

Gene expression analyses. For RNA isolation and reverse-transcriptase PCR amplification, osteoclasts preserved in RNA-later were subjected to mRNA

Table 1. *Real Time PCR examination demonstrates a dramatic increase in the expression of gene related to osteoclast activity and bone resorption*

	Arbitrary baseline value	Fold Change
	GROUND	FLIGHT
RANK	1	13,09*
Int β3	1	83,87*
Cath K	1	308,69*
MMP9	1	311,91*
ClC7	1	2,84*

*p<0.0001

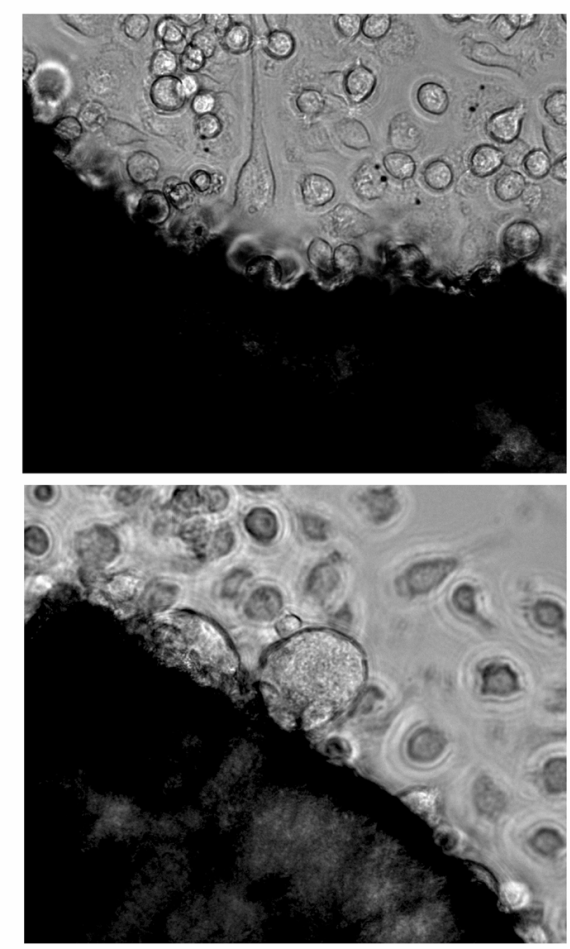

Fig. 3 *Tartrate Resistant Acid Phosphatase (TRAP) staining of osteoclast culture on a bone slice, prepared as control before slice integration in the bioreactor. Mature osteoclasts are visible on the surface*

with cells were fixed and stained to control the presence of mature osteoclasts, identified through the staining of Tartrate Resistant Acid Phosphatase (TRAP), an enzyme specific for osteoclasts (Fig.3). When, five days later the FOTON was launched the medium was changed. After four days in microgravity the cells were fixed with RNA-later, to allow RNA extraction after landing. Sections were thereafter fixed and observed to visualize the "pits" excavated by resorbing osteoclasts (fig. 4). Resorption cavities were evident, but could not be stained, due to the long time in RNA-later and RNA extraction. Real Time PCR examination demonstrated a dramatic increase in the expression of gene related to osteoclast activity and bone resorption, as cathepsin K, MMP9, CLC7 and integrin Beta-3 (Tab.1). The PITS experiment was designed to obtain gene expression information from resorbing osteoclasts in microgravity and the results observed, with a very high difference in fold

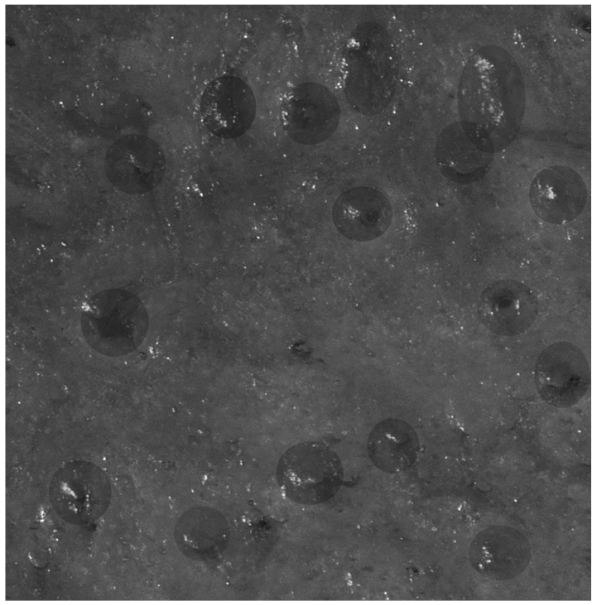

Fig. 4 *The microscopic observation of bone slice recovered after landing showed the resorptive activity of osteoclasts by pits formation.*

expression in comparison with ground controls after only 4 days of microgravity exposure, are striking. This very high fold increase can also be dependent upon the fact that proliferating precursors are always present in this kind of culture when MCSF is in the medium and the RNA obtained is derived from differentiated and differentiating cells. Undifferentiated cells, presumably more numerous on ground maintained bone slices, could be responsible for the lower basal expression level of relevant genes. These results for the first time show an activating influence of microgravity, and more generally of mechanical forces, on osteoclast activity, , at least in an in vitro system, supporting the hypothesis that osteoclasts have a critical role in microgravity induced bone loss. Possibly the weightlessness effects on isolated osteoclasts are stronger and faster in an in vitro system, where the cells are lacking compensatory mechanisms coming from the body. However these evidences indicate osteoclast as a direct target of microgravity and further address future studies in understanding the cellular and molecular mechanisms of osteoclast behavior in microgravity.

Abbreviations

Alkaline Phosphatase (ALP), osteoclast (OC), Chloride Channel 7 (CLC7), matrix metallopeptidase 9 (MMP9), α-Minimal Essential Medium (α-MEM), Fetal Bovine Serum (FBS), Macrophage Colony Stimulating Factor (MCSF), receptor activator of the nuclear factor-kappa B ligand (RANKL), tartrate-resistant acid phosphatase (TRAP), European Space Agency (ESA).

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