Human Spaceflight

Science Newsletter

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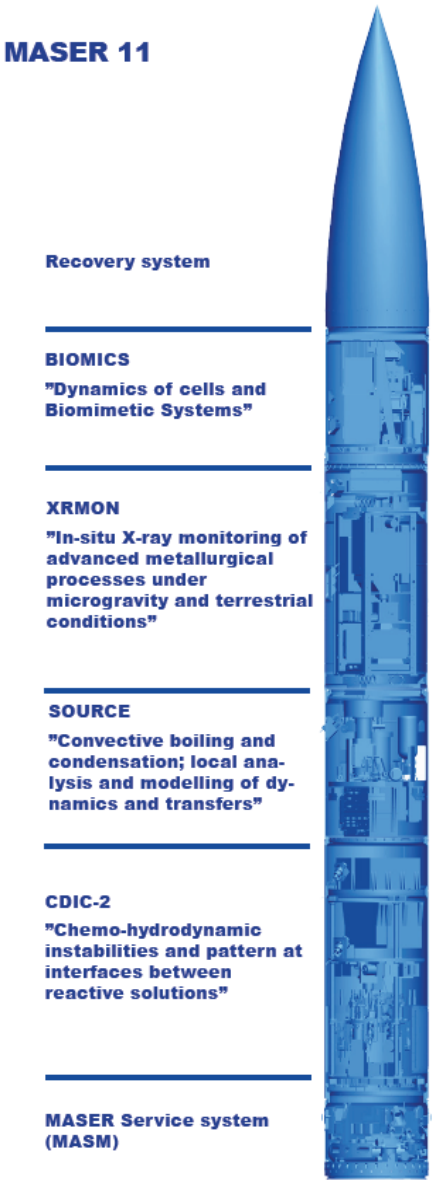
MASER-11MISSION: FLUID-, MATERIALS-, AND LIFE SCIENCES EXPERIMENTS FROM THE MAP-POOL

On 15 May 2008, 06:00 a.m. MASER-11 lifted off from the ESRANGE launch site near Kiruna, Sweden. The MASER Sounding Rocket Programme, conducted by the Swedish Space Corporation, offers a payload mass of up to 395 kg. The earlier MASER-10 mission was flown in May 2005, with experiments from two of the MASER-11 scientists onboard as well.

Onboard MASER-11 were 4 separate experiments, accommodated in the usual manner in four separate experiment compartments, stacked one on top of the other under the fairing. The earlier sounding rocket flight in 2008 - TEXUS 44 - was reported in Newsletter n°. 1, 2008,

Sounding rockets launched from this area, demand low winds and optimally snow-covered ground. The first ensures that the rocket lands still in Sweden (neighbouring countries Norway and Finland are only a short distance away), the second aspect for a smooth landing in the snow-covered landscape.
The rocket reached an altitude of 251 km and provided a microgravity period of some 6 minutes. In order to reach the apogee of 251 km over the Earth, the two stages of the rocket burned for a total of 44 seconds.



Right: MASER 11 Payload and investigator overview. Courtesy Swedish Space Corporation

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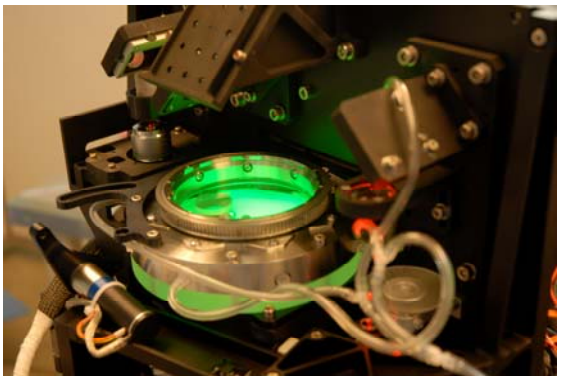
At engine shut-off the rocket decelerates until its vertical upwards velocity is 'zero'. Earth-gravity now allows for a free fall of some six minutes before breaking starts and the rocket touches down softly under the parachute in the still snow-covered northern Sweden.

The four experiments onboard MASER-11 are identified in the diagram above.

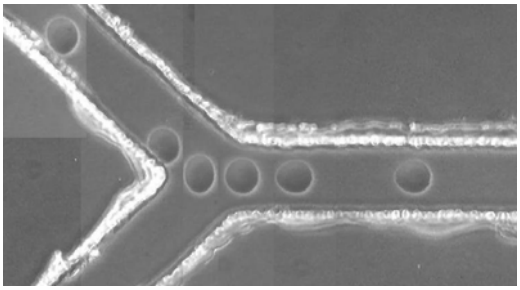


Shear flow chamber mounted on the Holographic Microscope (green backlight is for the overview camera) Courtesy of BIOMICS group

Experiment 1: BIOMICS – Bio-mimetic[1] and Cellular Systems

Selected under the Microgravity Application Promotion Programme (MAP) in 2004, the BIOMICS project is concerned with movement of vesicles, i.e. small, firm, but flexible bodies, in fluids.

With the help of modern techniques for monitoring of complex dynamic systems, the BIOMICS group has taken on as their ambition to try to elucidate how such fluid systems are influenced by gravity in particular. The case investigated is what takes place when a simulation of blood streams in a vessel, with its real *fluid phase* – plasma – being in it self a very complex blend[2], *and a number of different cell types*, red blood cells and the range of white blood cells and platelets. The setup remains a simplified simulation model to start with.



Evolution of the shape of a vesicle flowing through a bifurcation (mimicking natural circulation bifurcation, ed.) Source: BIOMICS October 2007 Report

This simplification of the real blood composition is in itself a challenging task when it comes to describing the flow dynamics on a detailed level. In reality the situation is much more complex: Cell surfaces and the lining – the epithelium - of the blood vessels, have receptor and activator mechanisms in the form of specialised molecules bound to the surfaces, which under normal conditions would attract and in some cases attack circulating cells. A simulation including the activity of such actors is the next step for the BIOMICS group.

Methods.

Instruments applied count a Shear Flow Chamber[3] and a Digital Holographic Microscope, which in the preparation phase for the MASER-11 flight have been used onboard ESA and CNES based parabolic flight campaigns.

The objective is ...." to obtain the 3D trajectory of each vesicle flowing in the shear flow chamber and passing the field of view.", quote from BIOMICS status report, October 2007.

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[1] The study of the structure and function of biological systems as models for the design and engineering of materials. Source: http://www.thefreedictionary.com

[2] .. of proteins, a water phase, lipids (fat), glucose, amino acids, and numerous tiny functional molecules e.g. hormones and vitamins in addition to waste products.

[3] A flow chamber where the flow paths are close to parallel. This arrangement offers standardisation of the flow parameters

One question asked in this context is:
How are vesicles distributed in an asymmetric bifurcation (see black and white illustration above) where downstream channel sizes and flow rates are different? (this is relevant for blood flow in capillaries)

A next step would be to artificially add adhesive properties to the vesicles, as mentioned above, introducing an additional level of complexity, but coming closer to the blood stream reality. Such experiments are planned to be performed in parallel with experimentation on "real" systems, i.e. organic circulation, in order to test the validity of the simulation model.

As an improved tool for analytical chemistry, a 'Hydrodynamic Acoustic Cell Sorter' is also being conceived – a system by which vesicles/cells of different size and density can be sorted in a moving fluid. By using acoustic wave emitters with different wavelengths and a wave direction across the fluid flow direction, cells will be guided along a narrow route, with a resulting sorting at the end of the flow path.
Preliminary results from the MASER-11 flight reveal a particular movement behaviour of the vesicles, by the authors named 'vacillating breathing mode' as a first investigation result. For technical reading on the phenomenon, click here.

The complete MASER-11 BIOMICS module. Courtesy of ESA

Lift: The mentioned experiment also shows that due to the fore-aft asymmetry of the compressible vesicles and the flow profile, cells tend to gather in the middle of the stream in the laminar flow, with some distance to the vessel wall, which involves a phenomenon called 'lift'. The vesicles used contain sucrose and the fluid around them mainly contains glucose, in an attempt to mimic the viscosity of blood. This construction leads to a 5-10% difference in specific density with the vesicles being denser. It means that the vesicles would sediment at very low fluid speeds, which is a useful effect that can be used to investigate the effect of different flow regimes.

The ultrasound cell sorting method is initially meant to be used for a sophistication of the research model, as the vesicles vary somewhat in size and therefore do not represent a single category, with same size, shape and interaction (i.e. not 'monodisperse'). Monodisperse solutions have the attractive quality, that a number of variables are removed from the calculation, as all vesicles can be assumed to be close to identical.

The objective of technically creating monodisperse solutions has large potentials in the pharmacological industry. MacoPharma, one of the industrial partners in this project concerned with equipment for blood transfusion and macrophage[4] research, allocates significant importance to this aspect for their research. In particular cell sorting is interesting. Cell sorting is of importance in the context of preparation of whole blood for blood transfusion. Standard clinical practises perform e.g. platelets harvesting from full blood by aphaeresis[5]. The blood is circulated between one's own circulation and an external separator in the form of a centrifuge, allowing separating components of blood with different densities. Platelets are essential additions to the

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[4] Macrophages are white blood cells involved as a scavenger cell in the body, and also form the precursor for diverse specialised cells, related to immune response and other specialised functions.
[5] Greek: Separation

blood of leukaemia and cancer patients, as even small bleedings in these patients, low on platelets, can become life threatening. In present separation techniques loss up to as much as 35% of the platelets is realised during the separation. That is considered too high, and is a part of the problem related to the necessity to remove as many white blood cells from the filtrate of platelets, as the white blood cells lead to so-called alloimmunisation in the recipient at repeated transfusions – a condition where the foreign white blood cells in the received blood stimulate an immune reaction against ones own cells, due to the contact with the immune defence of another individual.

Alternative cell separation methods are being investigated with the BIOMICS research.

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Experiment 2: XRMON - X-ray Monitoring of Formation of Metallic Foam

The foaming process has similarities with that of baking bread – Courtesy of H. Kropf

The XRMON experiment, another experiment from the 2004 Microgravity Applications Programme (MAP) pool was also performed successfully during the MASER-11 flight. On ESA's Parabolic Flight campaign 46 in November 2007, engineers from the Swedish Space Corporation, SSC, who is building the XRMON experiment module for MASER and who in particular has had the responsibility for constructing the X-ray observation system, tested the technology onboard the Airbus-300.

A further and equally important objective of that Parabolic Flight campaign was to test different candidate aluminium alloys for the final experiment onboard MASER-11.The Aluminium-Silicon-Cupper alloy, $AlSi_6Cu_4$ was found to be the best choice, considering the 6 minute microgravity period offered by the MASER rocket system.

The sample is preheated to around 350 degrees C before the microgravity phase begins, by means of an electrical coil system, with a melting temperature of around 700 degrees C reached at the point where the micro-g phase begins. The solid sample melts quickly and gradually starts foaming up, under the observation with the X-ray based system.

The diagnostic X-ray system consists of a micro-focus X-ray source, a CMOS[6] high-resolution digital x-ray detector and an image acquisition computer. Images acquired during the microgravity phase are stored on a solid state memory onboard.

After data has been received by the scientists, it will be taken through a conversion into something that can be read by their tailor made software.

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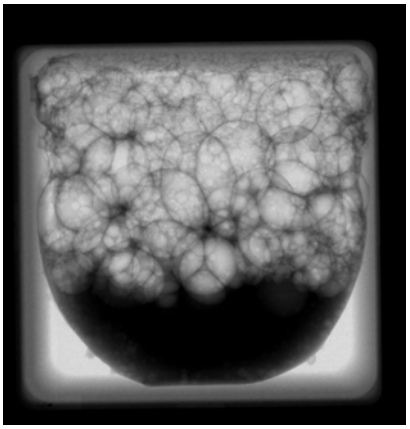
[6] CMOS: Complementary Metal Oxide Semiconductor – a chip-based image detection technique used in most modern Digital SLR cameras, parallel to the much used CCD that most video cameras apply presently. The CMOS has the special quality of being very low on energy consumption and uses a different data processing technique than the CCD.

In the analysis the focus is on the effect of temperature and gravity, evaluated via observation of bubble size, ruptures of the bubbles, relative distribution of ruptures between bottom and top, draining and timing of draining, as well as on the effect of temperature distribution. In terms of temperature control, the furnace used is probably the most accurately designed instrument for metallic foam processing ever built.

The samples are a mixture of the mentioned Aluminium-Silicon-Cupper alloy with some solid particles oxides that are supposed to serve as stabilisers of the foam when formed. When the initially solid material is being melted, it at the same time develops gas which travels through the melt and forms bubbles as the gas expands.

Experiment observations: The foam production industry has built their production facilities based on both a theoretical model and trial and error, until a satisfactory result is achieved.



X-ray 2D image of foamed-up Aluminium alloy On MASER-11. Courtesy of Garcia-Moreno

After the implementation of the X-ray monitoring technology was introduced, it immediately became clear that several of the details in the assumptions for how the process goes, were not quite what could be observed; the process certainly looked different than expected. This will eventually lead to correction of the definition of the best industrial production approach and equipment. As investment in new production equipment is however considerable, only when convincing qualitative and economical arguments can be made, would change in industrial approach be expected.

As the XRMON experiments have had just very few experiments to make their conclusions from, one could imagine that optimised choice of sample material and composition – via further sophistication of the model - still could be expected to lead to better materials in the future.

Applications: The metallic foam production is still a rather small industry with around 15 companies worldwide, producing and selling metallic foams, but already now they do find applications in the automobile industry, mainly as high rate energy absorbers in safety related constructions, with the purpose of protecting primary structures from taking the main blow of impact and crashes. Metallic foams have a number of very attractive qualities that are in the beginning of being discovered by diverse industries, but here are a few of them: The material

- is able to absorb energy over a wide range of forces, dependant on the construction characteristics
- is light-weight, 5-10 times lighter than bulk material (the solid equivalent of the alloy applied)
- is non-flammable
- is non-toxic

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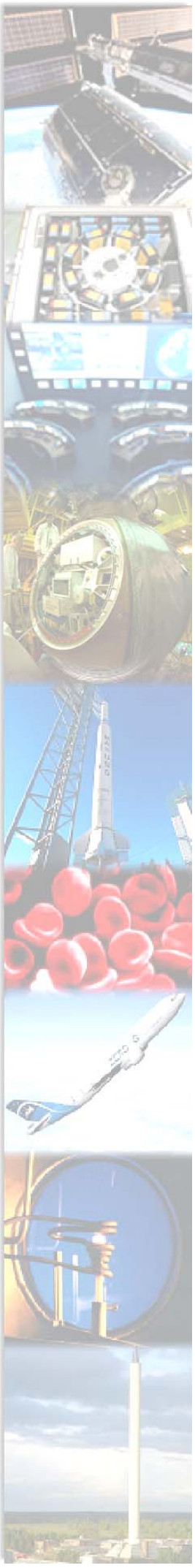

- is amorphous (i.e. has no specific shape) so that it can be made to take any shape in the solidification process, and could be seen as a contender to the role that today's honeycomb constructions have in light-weight constructions giving optimal stiffness

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Experiment 3: SOURCE or
Dynamic Heat Transfer Mechanisms in Flow Boiling and Condensation

The third experiment out of the Microgravity Applications Programme 2004 selection-round is concerned with the behaviour of fluids in tanks and as a part of overall MAP project 'Convective Boiling and Condensation'. More specifically, this is supposed to be a parametric study, to investigate the:

- Effect of convection on heat and mass transfer, in the form of local analysis at bubble and film scale, and
- Flow structures and pressure drop laws.

Boiling under normal gravity (lower pict) and under microgravity (top) on MASER-11.

By means of a transparent observation tank, the boiling process can be observed during the micro-g phase.

The fluid HFE-7000[7] is being used for the boiling experiments. The container has a linear axial temperature distribution which is achieved prior to the launch by heating the upper part. It is pressurized with nitrogen gas at 3 bar.
The bubble behaviour in microgravity is completely different from that observed under ground conditions. Without buoyancy, the bubbles remain in the vicinity of the walls. Experiments are performed at different settings of the heating level and under different liquid pressures corresponding to what in specialist terminology is called 'sub-cooled' or 'saturated' boiling conditions. The experiment is targeting investigation of the thermo-hydraulic behaviour of propellants for future cryogenic European upper propulsion stages.
Aspects of interest are prediction of:

- Location of the liquid in the tanks,
- Temperature distribution in the liquid and gas-phases,
- Amount of evaporated liquid and dissolved gas.

As illustrated in the images, gas developed during the boiling process behaves differently without the influence of gravity. On Earth, matter with lower mass moves upwards (here gas lighter than fluid). In microgravity the 'small' forces, e.g. surface tension, adhesion, relative pressure, heat gradients, etc. govern the behaviour of fluid and gas.

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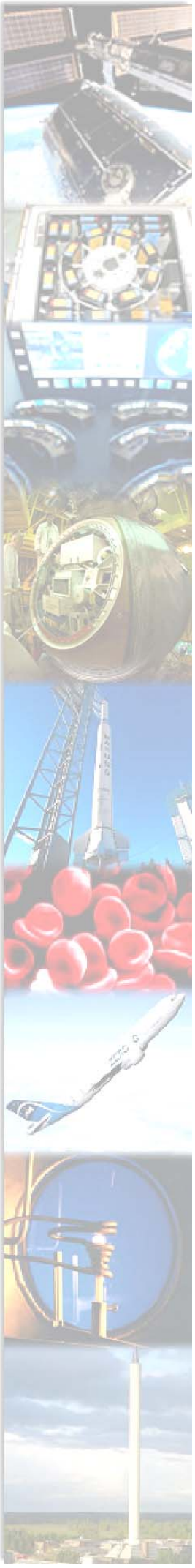
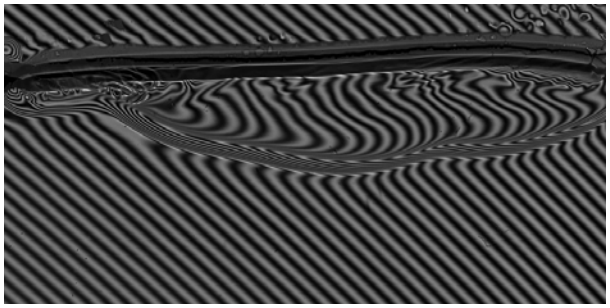
[7] HFE stands for hydrofluoroether, a so-called engineered fluid type, with very special properties, e.g. that is can be pumped at temperatures as low as -120 C and that it boils at a temperature of 34 degrees C, which makes it attractive for these experiments. It is also non-flammable, widely non-toxic and non-corrosive. HFE-7000 has the chemical formula $C_3F_7OCH_3$. The HFEs have come about as alternatives to global warming contributing perfluorocarbons (in particular CFCs), which have long atmospheric lifetime, i.e. are not broken down easily, which means they reach the upper atmosphere where they do harm. Source: Tuma et al.



Experiment 4: CDIC-2 - Chemo-Hydrodynamic Instabilities and Patterns at Interfaces between Reactive Solutions.

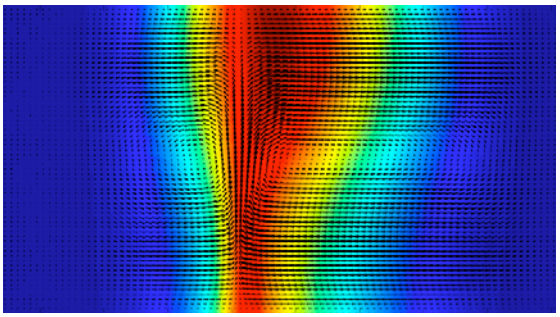


Interferogram showing the interplay between a propagating reaction front between two reaction fluids and a Marangoni convection. Courtesy of Kerstin Eckert.

Under this title a set of interesting experiments involving different fluids in contact with each other is hidden.
So why is this interesting, and important, one may ask? What is done and what can we learn?

The story begins in the broad production industry, in all places where for one or the other reason different fluids need to be in contact with each other in order to form a chemical reaction as a step in the production process.
The chemical industry has these cases *en masse*, e.g. extraction processes, in the food industry as well and one can mention almost endlessly cases where this is taking place. A very complex subject, when one tries to create mathematical expressions that will describe such processes: fluid dynamics in liquids influenced by chemical reactions and different forces - that is what CDIC science is about.

In order to understand why then, this needs to be investigated without the influence of gravity, give this a thought:



2D salt concentration field produced at the reactive front between acid (left) and base (right) solutions. Two counter-rotating vortices can be seen to develop.
The deformation is asymmetric because the base and acid solutions don't have the same density.
Courtesy of Anne De Wit

As an illustration, imagine two components in a fluid react to form a solid deposit at the bottom of the fluid: composed of some of the constituents of the fluid, the chemical product simply sinks to the bottom – it has become heavier than the fluid it came out of. Gravity works here.
A familiar example would be that of sugar in water– when dissolved it ends up eventually as a homogeneous fluid with equal density, although the solid (sugar) itself started out being heavier than water. When saturated, i.e. when no more can be dissolved, the remaining amount stays as a deposit. This serves as an example of how a fluid volume becomes denser by dissolution of a solid, even though dissolution as such is not considered a chemical reaction in a narrower sense.

To give a flavour of the reactions of interest for CDIC-2, remembering school chemistry lessons in which the progress of a simple neutralization reaction between an acid and a base was made visible by the colour change of an indicator is helpful. If we on top of an aqueous layer of a base, e.g. potassium hydroxide, which has a blue colour due to dissolution of a suitable indicator, carefully pour another aqueous layer containing an acid, the acid diffuses into the base solution and neutralizes the base in favour of the production of a salt. Thereby the initially blue colour of the base solution changes.

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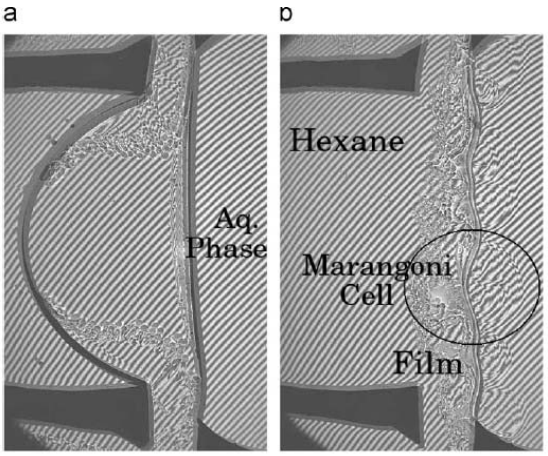
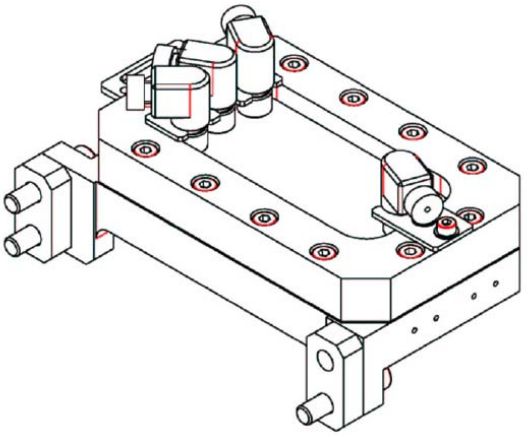


Fig. 4. (a) Filling of the IHSC under microgravity (Shi et al., 2005): the organic hexane phase (in which myristoylchloride was dissolved) was injected from the left-hand side towards the aqueous phase (0.1 M KOH). (b) Interferogram showing the coexistence of Marangoni cells (one of them is marked by a circle), wetting film and interfacial deformations at a stably pinned interface.



This is exactly what is seen on the coloured illustration above. In parallel one can observe the effect of gravity on the chemically driven process which drastically changes the shape of the reaction zone (in the middle) as well as the reaction front speed.
Performed under microgravity, any buoyancy-driven[8] convection effects due to concentration variation of the involved chemical species vanish and the reaction front speed becomes significantly smaller.

Immiscibility – that different fluids do not readily mix (the familiar oil and water example) – introduces with the liquid-liquid interface a new element into the system. Depending on the chemical species involved, the chemical reaction can now take place either at this interface or, after a transfer of a larger portion of the reacting species out of one fluid and into the volume of the other, across the interface, the chemical reaction can take place here as well. Furthermore, with the Marangoni convection (see 'Figure 4' above), which arises from local variation of the interfacial tension provoked either by concentration inhomogeneities or temperature change, another convection type beside buoyancy comes into play.

It is worth noting, that most of the reactions are exothermic, i.e. they release heat as an effect of the reaction, thereby changing density and interfacial tension which stimulates fluid movement as well. This Marangoni convection does its job also under microgravity. Hence it is able to supply the reaction front with 'fresh chemistry' so to speak, thereby accelerating the reaction front considerably.
Under terrestrial conditions, Marangoni and buoyancy-driven convection interact in a complex way with chemistry. A microgravity environment is a valuable tool to unravel the situation.
In this manner only, can the relative importance of these physical forces be observed, that pertain to the process proper.

In order to keep the process as observable as possible, so-called Hele-Shaw cells are being used for these experiments - a flat reaction chamber which is transparent (imagine two layers of a CD plastic cover, made water tight on all sides; this is pretty much a Hele-Shaw cell of a simple kind). The Hele-Shaw cell has the advantage that if the distance between the sides is small enough, that the fluid dynamics can be

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[8] **Buoyancy** is the upward force (relative to the directions of the g-vector) that a fluid exerts on matter that has a lower density than itself. **Convection** in the case of fluids can be defined as fluid motion brought about by an external force e.g. buoyancy or Marangoni shear stress.

observed in a so-called quasi 2 dimensions – a handy setup for description of such volatile experiments. The type now used is called the Integrated Hele-Shaw Cell, IHSC, right illustration above. Further qualitative detail of the reaction chamber can be found in *Chemical Engineering Science 63*, (2008) 3560-3563.

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References:

Ying Shi et al: A novel Hele-Shaw cell design for the analysis of hydrodynamic instabilities in liquid-liquid systems. *Chemical Engineering Science 63*, (2008) 3560-3563

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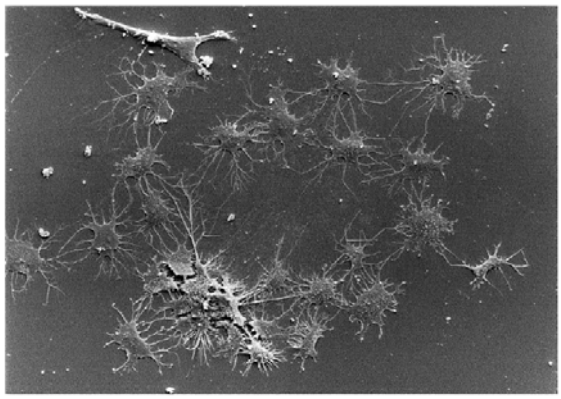
ESA'S BONE RESEARCH ACTIVITIES - BROAD SPECTRUM RESEARCH WITH A SHARP FOCUS



Scanning electron micrograph of a group of osteocytes. See further details later in article. Courtesy of P. J. Nijweide

ALMOST TWO DECADES HAVE PASSED SINCE THE FIRST SUBSTANTIAL OBSERVATIONS OF THE DECAY OF BONE QUALITY IN LONG DURATION SPACE CREWS. OVER THAT TIME INVESTIGATION METHODS, TECHNOLOGY AND DERIVED KNOWLEDGE HAVE ACCELERATED SIGNIFICANTLY AND THE MISSING PIECES IN THE PUZZLE BECOME FEWER AND FEWER.

BONE QUALITY, OFTEN FOR THE SAKE OF SIMPLICITY IDENTIFIED AS BONE DENSITY IS A PRODUCT OF THE ENVIRONMENTAL REALITY THAT TISSUE EXISTS IN, IN ALL LIVING ORGANISMS ON EARTH. LOADING AND ITS PATTERN IS WITH ALL LIKELIHOOD THE FAR DOMINATING FACTOR, BUT BONE TISSUE IS ALSO KNOWN TO A VERY HIGH DEGREE TO BE INFLUENCED BY THE UNDERLYING GENETIC PROFILE THAT EACH INDIVIDUAL POSSESSES – AND THE VARIATIONS ARE LARGE. DETRIMENTAL AS A SIGNIFICANT REDUCTION IN BONE QUALITY IS FOR LONG TERM SPACE CREWS, ESA IN CLOSE COOPERATION WITH THE OTHER SPACE AGENCIES HAS THIS TOPIC AS ONE OF ITS 'HOT SPOTS''

Introduction

This present text is one of three in this Newsletter dealing with 'bone' as a topic. Together these three texts have the character of an overview article, which tries to explain as clearly as possible, in layman's terms, why bone is so difficult to handle from a therapeutic point of view, what astronauts have to do with people who have bone diseases on Earth, and how to explain findings and observations. Each of the three can be read independently or together, as the reader should wish.

PRESENTING THE CASE

Bone as a mechanical system that carries and responds to loads.

That bones in our skeleton carry the loads we put on ourselves, including the loads of gravity on the surface of the Earth, is fairly evident to most of us. What is less evident is that bone also *responds* to the loads, and in a very active manner such that bone tissue is far more dynamic than most people are aware of.

From a mechanical standpoint, in particular the long bones in the body need to have qualities that resemble modern composite material, namely stiffness and strength, coupled with a certain degree of elasticity. When one or the other of these qualities decay, the bone is in trouble. If the strength is being reduced, for reasons we shall discuss later, fracture becomes a risk, from a sheer strength point of view, in the way that the bone no longer can support the same loads as usually put on it. If elasticity is lost, fracture becomes a risk, due the increased brittleness the bone expresses.

Thus, healthy bone is equipped to withstand extreme situations, both regarding loading as well as the forces put on it in terms of bending and torsion, having the tensile strength of cast iron, the ability to absorb and release energy twice that of oak, whilst it only has a weight equal to a third of that of steel – indeed an ingenious material. (Martin et al. 1989).

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Under normal circumstances, bone constantly reacts to the ever changing loads it is exposed to. It resides in a balance which will be demonstrated, explained and discussed in the following, a balance that can be disturbed severely, if the circumstantial conditions change.

A daily routine for living organisms in our gravity field consists of a wide variety of dynamic loads, as well as a significant amount of fairly passive situations, as when we sit down and rest or do tasks that do not require movement to any large degree. We also spend around one third of our time in the horizontal position. Extreme sports put high dynamic demands on the body as another extreme, where the bones are loaded far beyond what the average individual experiences.
All these conditions the skeleton can handle, and we do not note the adaptation that takes place, as this entire spectrum seems to be 'acceptable' to the normal healthy functioning of our skeleton. Living organisms are well adapted over millions of years to the Earth's gravity field - where we are under the constant influence of gravity.

Long duration spaceflights

The early long term human spaceflights revealed not only, that living under space conditions led to loss of bone mass, but also that the variation in response between individuals to this environment was remarkable. Some individuals were seen to loose very little, a few percent, over the duration of a 6 months flight, whilst in one extreme case as much of 20 percent of bone mineral at the start of the mission was lost after 6-8 months in space (Vico et al., The Lancet, 2000).
Methods for screening were macroscopic in character – mainly X-ray based - and did not allow investigating the underlying mechanisms in detail, even though the main key biochemical and hormonal actors were well known, and their so-called bio-markers, i.e. their metabolic waste products could be traced in the urine of the individual. These data alone did however allow to conclude *what* had been going on, namely that a disruption of the normally coupled bone formation and bone resorption seemed to take place in space crews (Caillot-Augusseau et al. 1998) but not how this had happened in detail. In relation to recent long term space missions, also an unexplained delay in recovery, manifested in an apparent ongoing loss of bone material even a few weeks after return from Space, has been observed. The fact that might offer a hint in that context is, that the capacity of bone-resorbing cells in removing bone material in one day, corresponds to what bone forming cells need 15-20 days to achieve. (Albright et al. 1987)

In recent years rapid progress has been made regarding revealing a large proportion of the information at the biochemical level, and significant insight into the cellular, molecular and latest, the genetic level has been achieved. On the molecular and genetic level a considerable growth in knowledge has shed more light over the intrinsic details of bone metabolism. Not the least modern techniques, lately the potential of use of chip technology for gene screening have accelerated the development, side-by-side with so-called 'knock-out' models[9] and antibody binding techniques. Nevertheless, there is still a long way to go.

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[9] Knock-out in this context identifies that, in the organism used for the test, one specific or more genes have been eliminated by gene manipulation. That has the remarkable effect that in cases where only that gene is responsible for an observable reaction to a certain stimulus, that reaction simply does not manifest itself any more. In 'clean' cases, i.e. when the entire effect is located to one gene, this allows in turn to conclude that that specific gene indeed was responsible for the reaction in question.

Bone loss on long duration spaceflights compared to disease

It is common knowledge that loss of bone quality is the main characteristic of osteoporosis, which is a significant societal problem. It is probably also a common perception amongst lay people that it is a disease for which till now therapies have been insufficient at best. That is to a certain degree a correct statement, but that situation will probably be seen to change significantly over the next decade, and maybe even significantly faster.

Relative to the characteristics and definition of osteoporosis, which we think it resembles the most, space induced loss of bone density has not yet been fully defined: Different diseases in the same organ system - as here pertaining to the skeleton - are normally differentiated from each other by how they manifest themselves. Thus, post-menopausal osteoporosis has a fairly well defined characteristic in, that there is an imbalance between the two processes, bone formation and resorption, leading to incomplete repair of the resorption pits that are continuously produced. In healthy bone these pits are fully 'repaired' by the bone formation process, with probably slightly less or slightly more bone matrix deposited than before the resorption took place, as a result of the 'request' that stimulated the process on that location in the first place.[10]

Space induced loss of bone mineral density is however a phenomenon observed in perfectly healthy younger individuals, and still needs to be characterized, not on the basis of disease as such but rather as a phenomenon that relates to a situation that we could call 'disuse' – which for comparison also seems to take place in muscle tissue. In addition, the changes taking place in Space have the character that makes us talk about accelerated loss of bone mineral, simply because the average rate with which we can observe it happening in space in healthy space crews, is uniquely high.

The overall picture at this stage

As will be seen from the following sections, ESA's activities are focused very much on the effect of gravity on development and maintenance of bone tissue, but as it happens, so are the activities of the rest of those who take interest in the fate of bone over the life time. Gravity acts via mechanical load, basically, and the big open question still is how this in detail is brought about. How are mechanical signals converted to cellular responses?

Research over the last decade has made significant progress, and at this time so much more is known than 10 years ago. Retrospectively, our focus has been the correct one for making progress, and recent experiments contribute to improving the situation. As can also be seen in the following sections, experiments indicate potential breakthrough in understanding the essentials of maintenance of bone, and with it the potential of development of smart approaches in the future that would allow space crews to maintain their bone quality with a minimum of time penalty.

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[10] This may be a cryptic sentence to the 'beginner' in bone matters: What is meant is, that if the stimulus to remodelling the bone is a constant reduction or a constant increase in bone loading, following the hypothesis indicated here, it would mean that less stress should lead to less material apposition and vice-versa. That is however the focus of the whole issue: What exactly is the reaction to which stimulus?

THE BOTTOM LINE OF BONE REMODELLING: BONE BEHAVIOUR IN LAYMAN'S TERMS

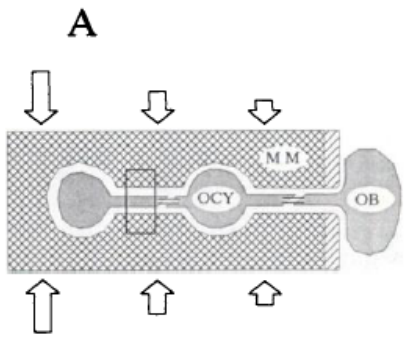

Diagram representing a current hypothesis of mechanotransduction in bone. Stress gradients produce strain gradients that squeeze fluid through the non-mineralized matrix (white) in osteocyte lacunae and canaliculi (see following figure for details).

Influence of gravity

The initial problem in the context of long term spaceflights is without any doubt the change in gravitational load. In daily life we do not subjectively feel that gravity is anything else than what we are used to, and therefore do not think of. But when in Space that load is gone, and thereby also the – rather constant – load on the skeleton, both our brain and our body sense the change.

As a consequence, the bone experiences this as a change that needs to be reacted to – or is it maybe the other way around? That, when the load is gone, the stimulus normally reacted to is gone? The drawing to the left illustrates what is thought to be the 1-g situation, namely that the constant pressure and loading of the bone (indicated by the arrows) stimulates the bone forming osteocyte ('OCY' in the middle) to react. This would mean that in microgravity, as the skeleton would not be exposed to load, the signals would be profoundly different.

So what is bone doing and how is it doing it?

Bone tissue is the body's slowest, in terms of material substance exchange, compared to all other tissue types – to the naïve observer probably even seen as material that does not change, once growth to adultness has taken place. But bone adapts continuously to the conditions it is exposed to and that adaptation probably happens with a high degree of accuracy, because despite of the physical appearance of bone, hard and compact, in terms of communication between cells a long distance away from each other, it is probably still as fast as any other tissue.

What we do know is, that a constant metabolism - here 'remodelling' in focus - takes place, where all surfaces of the bones react to local changes on loading parameters. The effect of these changes are miniscule, seen on a cell-by-cell basis, but considered as a system under normal circumstances, they cater for the appropriate preparedness of the bone to withstand the loads it is being exposed to, simply proven by the result in the long term, that very few of us experience fractures and bone problems during our lives.

Schematics of what happens

A fine balance exists between *bone formation* and *bone resorption* (production of bone matter as opposed to removal of bone matter). If that balance is disturbed, we either develop osteo*porosis* over time (fragility as an effect of too much resorption, leading to fractures, eventually) or osteo*petrosis*[11] (leading to very dense bones as a result of absence of resorption because of malfunctioning osteoclasts) – a condition that is likewise threatening.

So both extremes are problematic in each their way, which is one central dilemma overall and the reason why it is so difficult to handle the problem of 'bone loss'.

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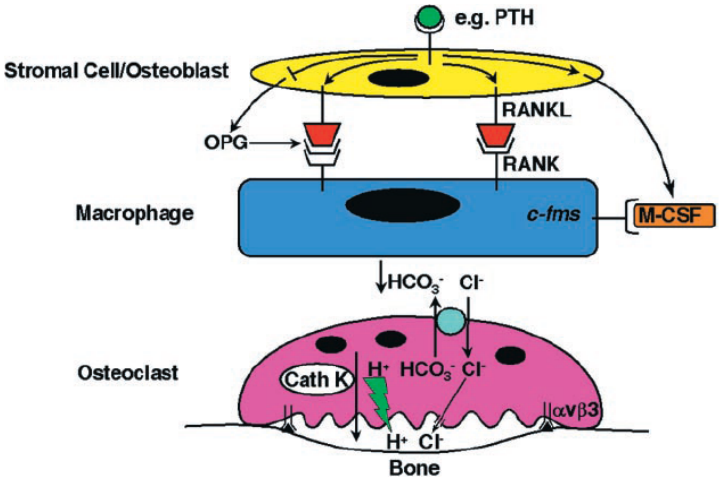
[11] from petroglyph: stone

A quick- and hopefully instructive and revealing tour through the bone system:

We have to do with the two processes with opposite 'sign', so to speak, the one that seems to be supposed to *form* and *conserve* bone via constant maintenance of maturating cells, to become mature bone cells; that process is called *bone formation* and involves osteo*blasts* and osteo*cytes* the first being the immature bone cell, laying down the solid matrix, the latter matured and reactive. And the opposite, with the apparent task of removing bone tissue from all surfaces of the bone; that is called *bone resorption*, involving osteo*clasts*. What they *seem* to be doing is however only half the truth, as this appears to be a very elegant teamwork under normal conditions.

In healthy bone, a balance exists between the two, which one indirectly can get a good impression of by measuring the concentration of calcium in the blood and urine. The blood concentration needs to be fairly constant. Excess will be excreted via the kidney, and a too low concentration will lead to mobilization of calcium from bone. So measuring concentration in the urine as well as in the blood at any point in time gives a fairly good impression of the 'balance', and normally the balance is there.

The cells involved – a closer look



In the figure to the left, borrowed from Steven L. Teitelbaum's review article in SCIENCE, VOL 289 , 1 September, 2000, the pink cell (colours used to distinguish only) with a ruffled lower limitation, called the 'ruffled membrane' is the active osteoclast and the blue entity above it, is where it comes from, so to speak. We will come back to that later. The particular appearance of the osteoclast is a result of a cascade of events, where one step is a precondition for the following, where a number of precursor cells end up having the appearance of a mature osteoclast. That cascade is illustrated in a later figure. If one step is incomplete, the functional osteoclast may not develop. The ruffled membrane configuration is a result of rearrangements internally in the cell, and the functional end-stage, with the interior cell skeleton, called cytoskeleton, playing a central role in that re-shaping. Once the ruffled membrane has been formed, the resorption can in principle start.

Take another look at the pink osteoclast: Three black dots indicate the nuclei, of which most cell types have only one. Osteoclasts have many, as a result of a fusion of a number of cells, namely the blue ones above it in the figure, the macrophages. In order to have that fusion of a number of cells going, the factor to the right of the blue, the M-CSF or 'Macrophage Colony Forming Factor', is necessary, just one example of a crucial step in the process. Without it, or if it is blocked, the cells will not fuse and no osteoclasts will be formed.

The yellow cell over the blue represents the bone *forming* cell line, the *osteoblast*, which actually as seen here, stimulates the formation of its 'enemy'', as it could seem, the osteoclast, via the different factors indicated. This communication is closing the circle, such that the development of bone resorbing cells actually is being dependant on signals from the bone forming ones, interestingly enough.

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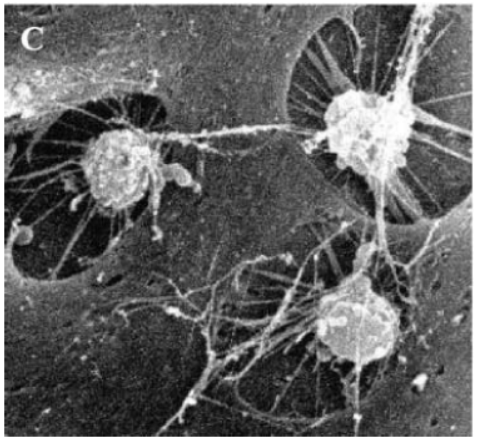
So why does this happen at all?



A fantastic capture in the form of a Scanning Electron Micrograph reveals individual osteocytes with cellular processes reaching out towards the lacunar wall and interconnecting cells in an intricate network. X 1600. Courtesy of Dept. of Functional Anatomy of the University of Glasgow.

Reasons why remodelling is done continuously

From a mechanical-philosophical view angle, bone represents a ridged tissue that has a crucial task to solve, namely the support of the overall integrity of the organism as a functional entity, a structure that needs to be optimally adapted to any unforeseen situation, in the short and long term. And it has to be of a light-weight construction in order to optimize the energy need of that moving organism. It is therefore designed optimally to satisfy these different, structural demands.

As other subsystems in the organism, body temperature and blood pressure as a few good examples, it could be working with a sort of set-point, or we could call it a 'target value' for that sake. This standpoint is supported by the fact that between species, there seems to be a strive in bone tissue towards obtaining a certain, fairly limited spectrum of strain, representing values between 2000 and 3500 microstrain (Rubin e al. 1984 and Nature 2001). These set-points or target values are probably regulated via local parameters - measured in 'physical load', pressure, torsion, maybe even frequency of impact – or 'strain', and experiments with readings from bone- attached strain gauges indicate that the forces produced by the muscles attached to bones may be sufficient as stimulus (same author).

Scanning Electron micrograph: Elongated resorption pits next to an active osteoclast. Courtesy of A. Zallone

As the skeleton seems to maintain its qualitative character in most individuals in relation to their daily routines, these impact parameters have a certain average value in normal life, which in turn creates an internal tension scheme in existing bone that is considered normal. And too high strain may have a destructive effect on bone.

How it is brought about is still the big open question. Looking at the osteocytes, however, and the way this active bone forming cell initially is suspended in its lacuna, and considering it being the far most abundant bone cell, the figure at the top gives the impression of a central cell body, the main mass of it being suspended in numerous attachment strings and thereby in contact with solid bone, as a multi-sensor accelerometer / communicator could be: Regardless of whether the reaction is to streaming potentials or to vibration brought about either by movement or by externally created vibration, these osteocytes seem ready to sense it all and transfer signals accordingly, and even to respond to direction variations. Being in a microgravity field, it is fairly evident that the stimulus they receive is orders of magnitudes smaller than on Earth. For sensor mechanisms in bone, see also Donahue et al. (1995) and Mosley (2000).

See an osteocytes in 3D here.

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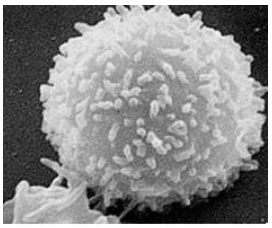
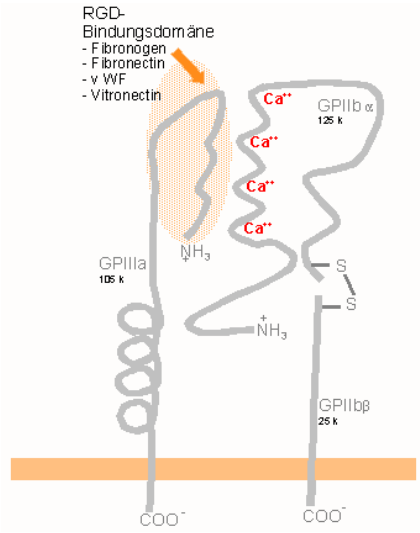
The resorption process



Looking again at the pink osteoclast, the limitation of the cell, indicating the cell membrane, has diverse chemical transport processes indicated, under which the important proton pump into the resorption space. The processes indicated here are those that have to do directly with the function of the osteoclast as a 'bone eater', degrading the existing bone. That is the process that takes place in the space between the ruffled membrane and the existing bone cell surface, as indicated. Osteoclasts start out being circulating cells and they need to attach to the bone in order to perform their job. Once attached, they close off the space limited by the ruffled membrane and the bone by forming a ring between itself and the bone surface, such that an acid milieu with a pH of around 4.5 (some researchers indicate even lower) can be created in that pit. That pH in turn is what is needed to break down existing bone with the effect that a so-called 'resorption pit' is being formed. For comparison, normal pH in blood is around 7.4, which value is regulated within a narrow span of less than 0.05 pH units. The 'anchor' that ensures that the cell lands and is held on to the bone surface is the one indicated in the second yellow box from the right in the figure below, the αvß3 – a so-called integrin, also indicated here to the left together with a full size white blood cell for comparison.

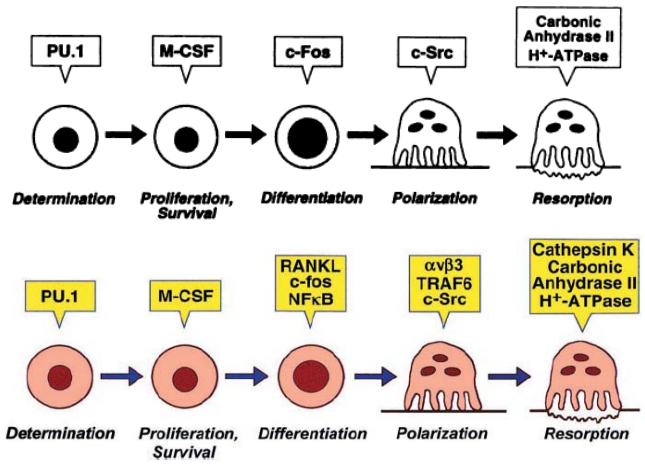


If that integrin is not materialising, the cell will not 'park'. The αvß3 receptor recognizes the tripeptide Arg-Gly-Asp (RGD – see figure) that is found in many bone matrix proteins located on the bone surface the osteoclast is supposed to 'work' on.

These cell surface binding and signalling factors come in a large number of variations, but always as a pair, one alfa and one beta subunit (see illustration to the right). They are located on circulating cells and involved in binding that cell to the polypeptide osteopontin on the bone surface.

Integrin (not αvß3), with the crucial RGD recognition zone that seems crucial for the osteoclast binding as well. Orange band = cell membrane. Source Juergen Bode For comparison an approximately 7 micron large white blood cell with receptor structures of similar size as the shown integrin.

Integrins perform this binding action in the present case between bone cells, but also in relation to for example formation of thromboses as well as binding of cancer cells, they play a decisive role. So they are a group of 'binding specialists' in the system.



Courtesy of Teitelbaum 1997 and 2000

Each integrin molecule complex typically binds loosely, such that there is a need of a certain concentration of these in a specific area of the cell surface, before the circulating cell will be bound. And to make matters even more complicated they are regulated up and down, i.e. their specific number per unit area changes, and probably very rapidly. Finally, to complete the condensed story about integrins, these molecules in the cell surface have both an extra- as a well as an intracellular component, so that they are dependant on what happens inside the cell as well as outside, and can transfer signals across the membrane, or change appearance and character as a result of same.

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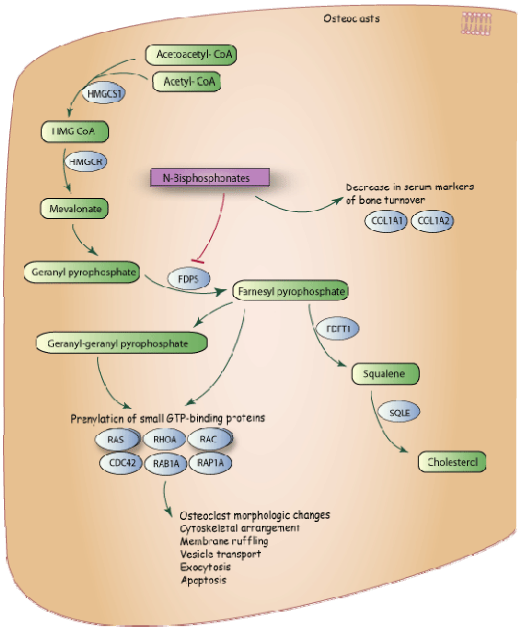
It seems as if the integrin variant αvß5 (meaning that the beta unit is different) starts the whole binding process, at the time where the fusion of the macrophages to form an osteoclast takes place. Afterwards, when the fused cell has become a reality, the variation αvß3 takes over and that integrin combination, the αvß3, has lately been found to have a particular folding of the large molecule, which may correspond to a "switching' mechanism by which the bone surface induces the particular osteoclast resorptive behaviour.

This completes the schematic explanation of how we come to a functioning osteoclast. The last figure above indicates in a schematic form which chemical and molecular factors or what one could term 'helper molecules' located at very specific places with very specific tasks are necessary for the development to mature osteoclasts. Between the black and white and the coloured version of the drawing, three years have passed, the first one from a publication of Teitelbaum 1997 and the second from year 2000. One notices that the earlier model contains less factors in the accompanying boxes than the later one, probably as an expression of the evolving understanding of which factors play a determining role for the cell development.
Absence of one of those factors will lead to complete or part abolishment of the cell development. Thus lack of the first factor to the left, PU.1, is lethal. The further we move to the right in the figure the less severe the impact is for development of the osteoclast - if for example the αvß3, the binding integrin is missing, the cell *will* be formed and prepared till the point where it should attach. But it cannot attach and resorption will not take place. For each step the determining accomplishment of the cell status is also indicated.

All the factors indicated in the yellow boxes and many more, but less prominent, not identified here, are in principle locations that one could consider as therapy intervention points, in terms of trying to influence the bone formation-resorption balance. The drug of preference for the time being is still to a large extent the bisphosphonate group, which in general terms has the effect that it stimulates osteoclasts to die prematurely, thus inhibiting the resorption. But according to the theory of how quality bone is formed, it will eventually lead to an impairment of the bone quality.



Effect of Nitrogen containing bisposphonates,
Source: http://www.pharmgkb.org/

The formation of bone takes another route and the two cell types also have different origin, which we shall not elaborate on here.

Therapy - Effect of Bisphosphonates

Bisphonates have different effects – there are a handful of different types of these available - depending on whether the specific type Bisphonate molecule has short side chains, in which case they are metabolized by the cell and the building stones incorporated in the basic nucleotide turnover in the cell, or have long side chains, in which case they are not metabolized. The effect is either that they induce cytotoxic effects on the osteoclasts or are inhibiting vital processes for development and function of the cell.

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Basically two main types of this drug type exist, those that contain nitrogen (purple in figure to the left) and those that don't. The non-nitrogen containing types generate an analogue compound to the essential energy transporter in the cell, the ATP, which eventually replaces ATP in the energy production in the cell, with a resulting cell (osteoclast-) death.
The nitrogen containing type, which is more potent, exerts its effect simply by blocking a central enzyme, which in turn blocks a row of enzymes secondary to that process. This in turn also leads to osteoclast premature death.
The immediate effect is that osteoclast development becomes severely reduced, leading to less bone turnover.

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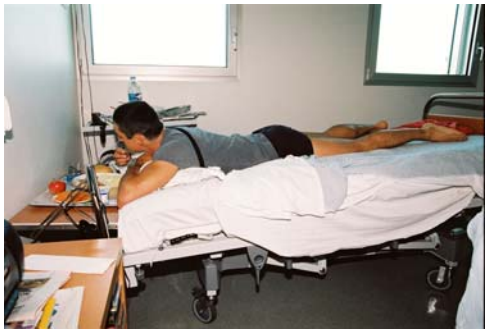
LOOKING FOR FACTS - ESA'S PARALLEL BONE RESEARCH ACTIVITIES

Introduction

As has been seen in several areas, where Space travelers present particular health-related problems, scientists engaged in the problem-solving energize a considerable focus on a specific issue that otherwise might not be given the same energy, research-wise.
As an example, Space based research is contributing with very important discoveries in elucidating underlying mechanisms for developing hypertension (Space observations in humans make it mandatory to seek a better understanding of the salt and water household, an aspect central to hypertension research), and here in the present context, we also would expect eventually to be participating partners in solution finding related to loss of bone and bone quality.
Thus, the sharp focus on the impact of loading and non-loading effects, that are so central to living in Space has been kept over the years, with the objective of finding cures against the loss of bone mineral density and bone quality, as observed in all astronauts on long term Space missions. This focus is guiding us around into different research initiatives on all levels of the problem, from system to gene, and should be seen as the background for the activities described in the following.

Bed Rest studies – first series after 2000



Bed Rest studies are performed as simulation of low gravity. Presently a duration of a few months is used, which is considered sufficient to create similar effects as long term spaceflights. Bed rest studies *are* simulations and mostly create effects on the tissue, that only partly mimic what happens in weightlessness. These studies are however very useful for bone studies, because significant offloading of the bones happens and creates a similar pattern as seen in Space crews.

ESA is continuously performing these studies in close collaboration with the international partners, and has over the last 6-8 years taken the lead in that respect.
Loss of bone mass in study subjects shows the same trend i.e. takes place in the same regions as observed in-flight are affected, but the particular quality of the studies is, that different therapeutic approaches can be tested, unlike what would be possible in space crews.

The first long term study in the newer era, a three months study with healthy young men took place around 2000-2001 and was a great success in many respects. Bone loss was observed and measured, and – importantly - countermeasure medication in the form of Bisphosphonate administration was applied too, in one group, whilst another performed exercise a number of times per week, as a means of loading muscles and bone.
Bisphonates, with a marked ability to prevent bone resorption, by hindering recruitment and growth of osteoclasts (see earlier article here) are a class of synthetic pyrophosphate analogues, pyrophosphate being a physiological compound naturally playing a role in regulating the calcium metabolism in the body.

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Bed Rest studies – next series after 2000

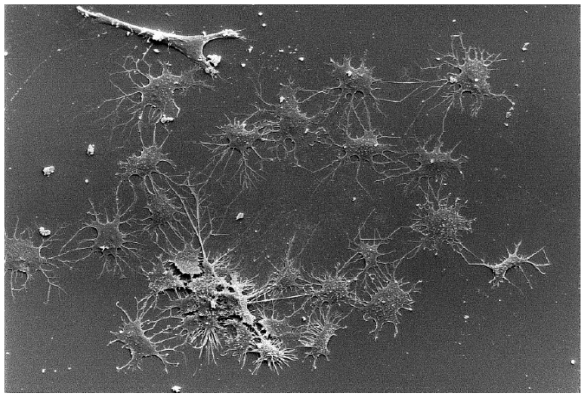
The next generation of bed rest studies involves vibration of the muscle and bone system, an approach that involves loading with consideration of vibration energy and frequency, as indicated in other studies in the 1990ies to have a significant importance.
How much and how often vibration needs to be applied in order to generate a positive effect is still being disputed, but a large number of publications exist, which demonstrate beneficial effects of very low impact with frequencies between 10 and 15 Hz. On the other hand, a wide spectrum seems to be applied by different groups.

Both NASA and ESA support studies in this respect, for the perspective that astronauts – and bed ridden patients likewise – at some point in the future would be able to maintain their bone mass, and structure of same, almost intact via minutes of such stimulation a number of times per week. (Rubin et al., several publications).
The potential here is considered to be significant.

Biological samples in space - KUBIK and FOTON

In the intact organism, one can study an intact, 'well oiled machine' to use a metaphor with some merit to it: In living organisms one has the advantage that all necessary components are there and in appropriate amounts. The disadvantage is that our options for invasive measurements are limited.
In tissue sample preparations, one needs to be sure that the necessary milieu is provided, which otherwise could lead to faulty conclusions. The clear advantage is however, that the degree of freedom of intervention is rather unlimited.

At several occasions bone samples have been flown on ESA missions, latest as a major part of the experiment complement onboard the FOTON-M3 mission in September 2007. As well, we reported on experiments flown in the KUBIK facility. In particular the PKINASE experiment, described in the November 2007 Newsletter can be considered related to the present discussion of bone related activities.
The experiments on bone samples flown on the FOTON-M3 mission had the objective of gaining knowledge about how bone *in vitro* responds to (off-) loading. This research is complementary to observations from experiments performed with the crew, and to the ground simulation bed rest studies. It is indispensable as they give information on the cellular, molecular and genetic level, which is supporting observations in the intact system. Only in this manner can one hope to improve the understanding of events. A preliminary science report on bone experiments onboard FOTON-M3 is given later in this Newsletter.



Scanning electron micrograph of a group of osteocytes in a 2D preparation. The cells have re-established a cellular network by moving away from each other and making thin, branching cell processes that connect with those of neighbouring cells. Micrograph kindly provided by Dr. P. J. Njjweide. *FASEB J.* 13 (Suppl.), S101–S112 (1999)

Loading experiments with mice – the effect of vibration

Loading and gravitational effects are without any doubt the central issue regarding conservation of bone, in particular when the talk is about healthy bone in healthy subjects. But how are they brought about?

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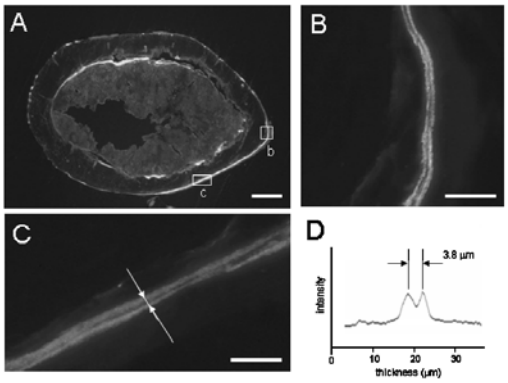
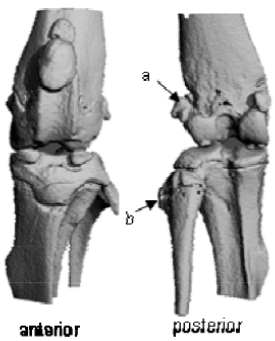
Since beginning of the 1990ies many attempts have been made to improve the understanding of this difficult matter, and several theories were put forward. One of the original publications frequently cited is the one on the shear flow theory, which still is one of the leading theories.
It was put forward by among others Klein-Nulend and Burger (1995) in a paper on how bone cells respond to stimuli via biochemical pathways. That paper was followed up by another overview paper in 1999 in the same journal, from where the electron micrograph above is taken. The group of Burger and Klein-Nulend were very much involved with ESA in the early attempt to decipher the effect of loading or absence of same on bone, relating to Space crews.
A more recent paper, investigating the effect of transverse loading to joints, more specifically in mouse hind limb knees, finds that that bone is twice as sensitive to that form of stimulation as to loading in the longitudinal direction.
This discovery gives a slightly different perspective to bone loading - that loading transversely, and in the growth zone, seems to have an amplified effect compared to longitudinal loading.
What this also tells us is, that forces that have to do with bending and joint movement maybe have an even stronger effect than the loading along the long axis of the body.



Two Hz mechanical stimulation of a mouse knee for 3 minutes in 3 days. Tanaka et al. 2004

The growth seen in a transverse section of the mouse bone in the figure to the left is a result of 3 minutes of oscillatory stimulation per day for three days, and in addition, this group finds support for the theory put forward by the Burger group, by measuring the so-called streaming potentials in fluid pertaining to bone canals.
The figure illustrates the growth as a result of low level loading with a oscillatory frequency of 2 Hz, seen as the space that separates the two lines (fluorescent dye, calcein strips). The space between the arrows corresponds to growth as a consequence of the loading. The opposite knee, not stimulated, served as control and did not show any growth of that magnitude.

However, a word of caution here: The way to read this, is in the *principles*, namely that 'short term, oscillating mechanical loading with a certain frequency and low energy, seems to be a potential way to maintain bone mass and maybe bone quality even in humans'. This, therefore, initially serves as a stimulus for further research.
Having said that, however, Rubin et al, have performed a massive amount of research to this end in humans and there are clear indications of a very positive effect of this kind of bone stimulation.



Zhang et al, *BMC Musculoskeletal Disorders* 2006, 7:73: Result of 3D reconstruction of a mouse elbow by Desktop micro-CT by SCANCO, Switzerland

Finally, as a reminder of the engagements that ESA takes in the field, the group of Tanaka, who produced the date described above, used another device developed under an activity that ESA has been involved in via the Microgravity Applications Programme (MAP), namely the Desktop micro-CT for 3D reconstruction of bone structure with a resolution of 30 microns, developed by SCANCO, Switzerland. Related to this development ESA was sponsoring participating scientists under the MAP heading. We now see more and more papers presenting very useful image data from this device-line.

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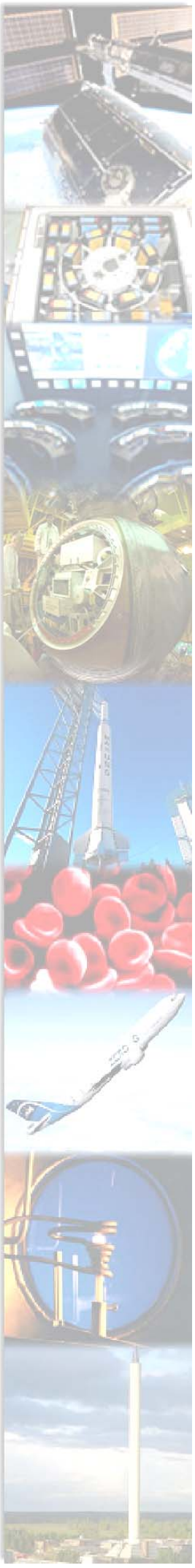

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Zhang et al (2006): Knee loading stimulates cortical bone formation in murine femurs. BMC Musculoskeletal Disorders 2006, 7:73

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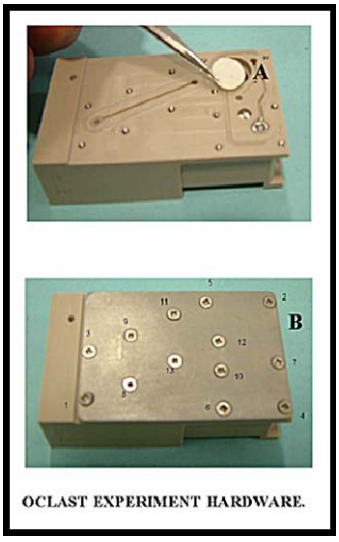
FOTON-M3: PRELIMINARY SCIENCE RESULTS – BONE AND OTHER EXPERIMENTS

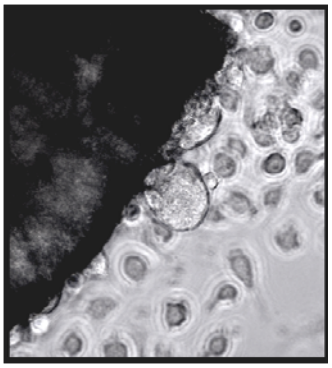
Onboard the FOTON-M3 mission, in September 2007, a number of bone-related experiments were flown. The September 2007 Newsletter, see p. 6 of that issue, gives an overview over experiment names, and in particular some of the living species flown in BIOPAN, and in most cases a link to the underlying justification of the experiment is provided.
Those ESA sanctioned bone-related experiments that reported data at the first Post-flight Science Review are described in the following indicating as far as available the preliminary findings. Reporting on the multitude of other FOTON-M3 experiments will take place in the following Newsletters.

OSTEO:
(McGill University, Montreal, Canada – R. Gorczynski)



OCLAST EXPERIMENT HARDWARE.

This is a typical example of an *in vitro* bone experiment, with bone samples embedded in diverse solutions of process activators or necessary process compounds, with the objective to see if the exposure to microgravity changes the cell response, compared to 1-g. This is measured via a number of the factors and receptors that can be investigated by identifying their appearance on the surface of the stimulated cells. To a large extent, fluorescence techniques are used, as these enable marking the smallest molecule entities by binding of a marker molecule that fluorises under certain light conditions. Another technique, and a fairly classical one, is the application of staining where molecules searched for have specific affinity for the stain applied.
In particular this group has been focusing on a novel factor, playing a role in the process of fusion of macrophages as a precursor for formation of osteoclasts (for details and explanations to this, see 'The Bottom Line ...'section, earlier), the CD200[12], strongly expressed in macrophages at the times for cell fusion to start, and is one in a row of known factors necessary for a flawless process. It is now understood that, some of the same factors that viruses initially need for the typical cell fusion process (where both an attachment mechanism and a fusion peptide is needed) seems to be true for the osteoclast development as well. Finding such similarities often improve the understanding of the process involved.



CD200 seems to play a role at the onset of the cell fusion where it is first observable. Samples from wild type, i.e. natural animals are compared to so-called 'knock-out' version, which consists of material where the essential gene(s) have been manipulated a priori, such that they cannot be activated upon stimulation. CD200 deficient individuals have a higher bone density compared to controls, as an expression of reduced bone resorption.

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[12] 'CD' in this context stands for Cluster of Differentiation, which is a system for identification of cell surface molecules present on white blood cells. They can have many different functions, e.g. signaling, cell binding, antibody binding. There are more than 250 such CDs known.

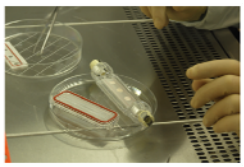
OCLAST experiment BIOBOX 6

(University of Bari, Italy – A. Zallone)

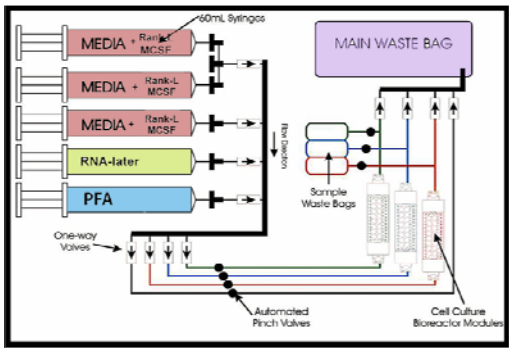
In the OCLAST experiment, mature osteoclasts were cultured on bovine bone slices under accurate temperature control. Embedded in an appropriate culture solution, the amount of bone resorption could be judged from the amount of one of the essential 'products', the collagen telopeptides, indicating that break-down of bone matter has taken place. On the microscope picture (black and white above), active osteoclasts are seen as circular and semicircular excavations in the border of the bone. An additional evidence of increased osteoclast activity was that the number of excavations pits per unite area was increased as compared to ground controls.
Also RNA was obtained from genetic screening, further supporting the claimed findings.

OSPACE – Osteoclastogenesis in Space

(University of Bari, Italy – A. Zallone)

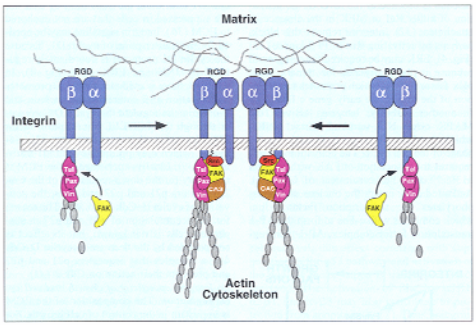


In another experiment this group attempted to stimulate what is called osteoclastogenesis, or simply formation of osteoclasts from a culture containing the crucial cells and factors.



Designed by one of the leading groups in the world regarding keeping osteoclast colonies 'alive', sample chambers containing slices of bone have been prepared in a fluid flow-through system, enabling 4 different solutions to flush the chambers, and there after to be deposited in a waste bag.
As can be seen from the schematics, the solution in addition to the media contains selected factors, which we recognize as some of the central ones to be available, in order to stimulate formation and function of osteoclasts, see section on 'The bottom line...' in this issue. One 'Cell Culture Bioreactor Module' is seen in the small insert..
A synthetic 3D bone-like material, skelite, has been used as medium for the enclosed osteoclasts. Skelite partly represents the biochemical composition and structure of natural bone. Initial data suggest that the markers indicating osteoclast activity and binding to the skelite material is markedly higher than in ground control samples. At a later point in time, micro array screening (DNA chip technology) will be performed.

Results of this experiment are very clear, as all the markers for increased osteoclast activity in the Space samples, compared to the ground controls are significant. More on detailed data on this experiment will be available later.



CONNECT – BIOBOX-6

(University of Liege, Belgium – B. Nusgens)

This experiment investigated the details of the adhesion of the osteoclast to the bone surface. The details investigated relate to the binding of the osteoclast to the bone surface. In the figure to the left the external surface of the osteoclast

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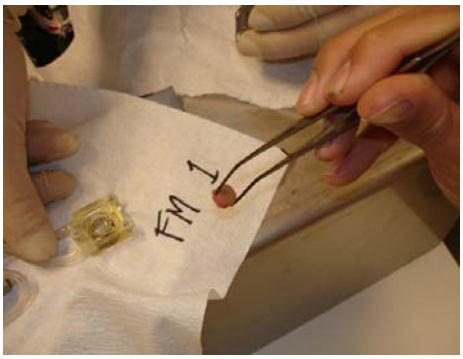
with its 'binding specialists' the integrin αvß3 (see the section 'The bottom line...') on the outside (top) of the cell, with the intra-cellular smaller part of the integrin interacting with the cytoskeleton, which has a profound impact on the cell shape changes that take place in the maturation process. The more colourful entities are all factors that are essential for activation of the cytoskeleton in the process. You will also recognise the letters RGD which is the amino acid sequence of three amino acids that integrin is assumed to need in order to attach. So the orientation of the figure is the intact bone, in the process of being 'attacked', at the top of the figure, the red-yellow components being situated inside of the osteoclast cell.

Conclusions:

- Cell area in microgravity is reduced
- Number of stress fibres in microgravity reduced
- Further conclusions will be provided later

FREQBONE

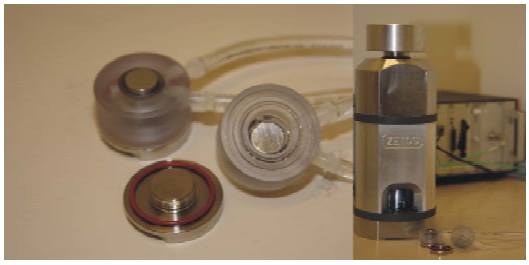
(University Leuven, Belgium, University of Marburg, Germany, AO Institute, Davos, Switzerland – Bioreactor: D. Jones)



A milled-out bone sample of fresh bone being placed in the incubator

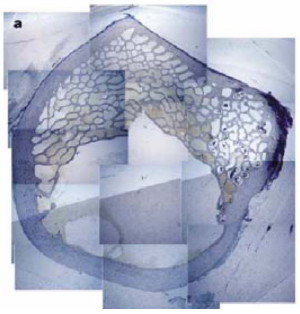
This experiment has been investigating the effect of microgravity on fresh bone samples, which in principle have had the entire reactive osteoblast-osteocyte-osteoclast system intact. High-frequency and low impact vibration has been given to one set of samples, in order to compare the effect on passive samples with samples exposed to vibration.

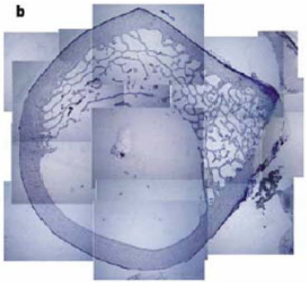
As indicated in the section 'The Bottom Line ...' earlier in this Newsletter, vibration is of particular interest for maintenance of bone quality in low gravity fields. In this experiment, a frequency of 30 Hz has been applied, one loading session per day, with a dynamic load of 3 Newton.



The Zetos bioreactor for culture and mechanical loading (D. Jones)

The experiment basis is partly on the background of one central publication in Nature 2001 by Clinton Rubin et al. reporting on the effect of low-magnitude, high frequency vibration as an osteogenic (i.e. bone forming) stimulus. Rubin has been referenced in the section 'The bottom line...' as well, for his vast work in the area of characterising the effect of loading patterns on bone. The perspective and the driver for this experiment has also been indicated in the same section, namely, the perspective of using a limited amount of time in conserving bone quality in space. His two illustrations from that paper are presented here as well, where an improvement of the bone status in the stimulated bone is visible. The effect of this is that sheep bone of daily stimulus is led to 32% more bone material over one year (lower picture to the left)





In the FOTON-M3 Freqbone experiment the daily 'dose' was 10 minutes of 3 Newton load, on top of a 'pre-load' of 30 Newton. These

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values have been chosen based on what degree of impact or compression of the bone tissue is targeted. Elsewhere in this Newsletter we have indicated that a level of 2000-3500 microstrain would be appropriate (Strain: The degree to which a material is compressed or extended. Strain is dimension-less) Standard labelling of bone formation fronts were used, calcein before flight and alizarin (green and red dyes respectively), an early stage indicator of new bone.

Analysis of the harvested samples is still outstanding at this time. The upper image shows 32% more trabecular bone after long term daily 20 minutes stimulation, as compared to age-matched controls (lower image). Even a strengthening of the trabeculae, in terms of cross sectional area could be observed, indicating maintenance of structure and strengthening of the structural elements. The author argues that loads as low as 5 microstrain (compare to indicated values above), corresponding to passive standing, may be enough for minimal bone tissue maintenance stimulation.

Reference.

Rubin, C. et al. Nature, vol 412, 9 August 2001

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