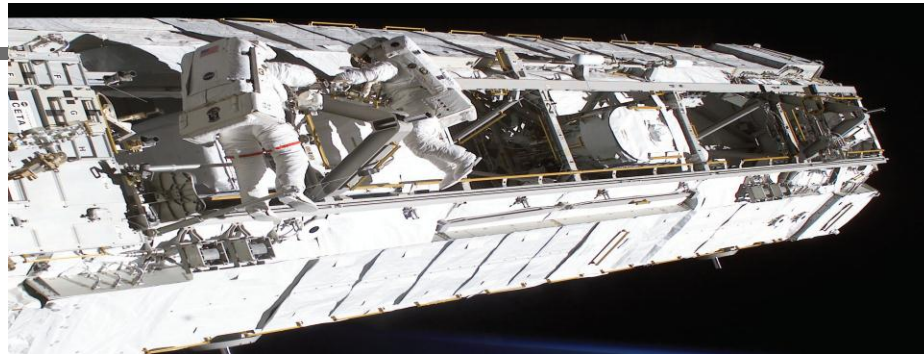


Space radiation and osteoclastogenesis: The effects of radiation and microgravity on bone resorption:



Alamelu Sundaresan¹, Sukesh Aghara², Terrell Gibson¹ and Indi Siripirasan²

1: Texas Southern University-3100 Cleburne Street, Houston, TX.

2: Prairie View A and M University, Houston, TX.

Contact: sundaresana@tsu.edu



Abstract

- Cellular three dimensional bone constructs (Dr. Sundaresan 2005, ECTS 2006, ECTS 2007,2011 Sundaresan US Patent **8076136**) cultured in the High Aspect Ratio Vessel (HARV) vessel (modeled microgravity) and measuring resorption with and without space radiation. Preliminary results indicate that osteoblasts (bone forming cells) are suppressed and osteoclasts (bone resorbing cells) remain alive longer and are very active for very long periods in the microgravity analog environment. Low doses and high doses of gamma and x-ray radiation will be tested on this model after fibroblast testing to assess any synergism with radiation exposure. Radiation works synergistically with some organ systems especially in relation to the inflammatory and immune response in organisms. Hence it will be important to assess the effects of microgravity combined with radiation exposure in bone tissue. The readouts used will be key bone homeostasis modifiers, such as Tartrate resistant acid phosphatase (TRAP) staining, receptor for activated nuclear factor kappa B and its ligand i.e., RANK=RANKL interactions, Osteoprotegerin (OPG) expression and the fate of other mechanotransduction pathways. The effects of dose and cell function assessments such as apoptosis or cell death, bystander effects and bone cell proliferation will also be assessed for a potential numerical model. This model will address both the synergism of radiation with microgravity and the relative kinetics of osteoblast function and osteoclastogenesis in microgravity.



Premise

- Humans traveling in microgravity experience a 1-2% loss in bone mass per month. Models for bone loss are essential to exploration. Presently we investigate humans on ISS, subjects in bed rest protocols and changes in cellular events within bone. A rotating cell culture technology created by NASA affords the only cell-based microgravity analog to investigate the cellular, molecular and genetic basis for loss of bone that occurs in microgravity. We hypothesize that there are significant changes in the signaling among bone cells and surrounding tissue that occur in microgravity. We will use the analog culture system as a model and a test bed to determine the signal pathways relevant to bone loss in microgravity. Bone loss is a type I risk in the critical path roadmap.
- Bone is made up of several different cell populations. Osteoclasts are responsible for the breakdown of mineralized bone, in preparation for bone remodeling. In contrast the osteoblasts synthesize mineralized bone in the remodeling process. The goal of this project was to **develop an “*in vitro*” three-dimensional, cellular model of osteoclasts and osteoblasts (human and rodent) cultured together in microgravity analog culture conditions to identify the underlying biomarkers related to bone loss in microgravity and the cellular mechanisms involved in bone resorption.** The NASA Rotating-wall Vessel (RWV) permits the growth of mixed cell cultures for much longer periods than traditional culture methods. This would set the stage for development of countermeasure strategies for bone loss in space as well as in osteoporosis and rheumatoid arthritis which are increased health risks on Earth.



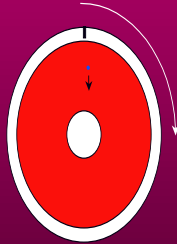
Objectives and Methods

- **Objectives:**
- To develop an “in vitro” cellular model of osteoclast and osteoblast interactions to facilitate investigation in microgravity analog culture conditions. The Model will consist of analog microgravity (NASA RWV) co-culture system of primary human osteoclasts and osteoblasts.
 - Determine culture conditions for maintaining osteoclasts under RWV culture conditions.
 - Determine culture conditions for maintaining osteoblasts under RWV conditions.
 - Determine RWV conditions required to co-culture osteoclasts and osteoblasts in the same construct while promoting differentiation of both cell types.
 - Preliminary radiation experiment with fibroblasts and assess radioprotective potential of hydrophobic extracellular matrix proteins such as amelogenin (Reseland and Lyngstadas 2012)
- **Progress:**
- Successful growth and differentiation of human primary osteoblasts on collagen-coated beads under both static and RWV culture conditions.
 - Osteoblast cells produced 3D constructs when grown on beads. Cells produced the early differentiation marker, osteocalcin (OC), under both culture conditions and significantly more of the late differentiation marker, alkaline phosphatase (AP) after 7 days of culture in the RWV relative to age-matched static control cultures.
 - Expression levels were compared utilizing immunofluorescent staining followed by confocal microscopy under identical imaging conditions-see back up slides.

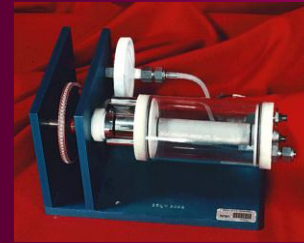
The Model

RWV Bioreactor Theory

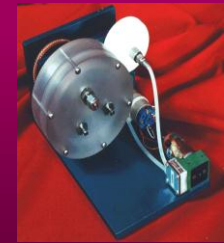
- Solid fluid body rotation
- Sustained free fall of cells or particles through medium
- Randomized gravitational force
- Relative low shear forces



Rotating Wall Vessel (RWV) Bioreactor



Slow Turning Lateral Vessel
STLV

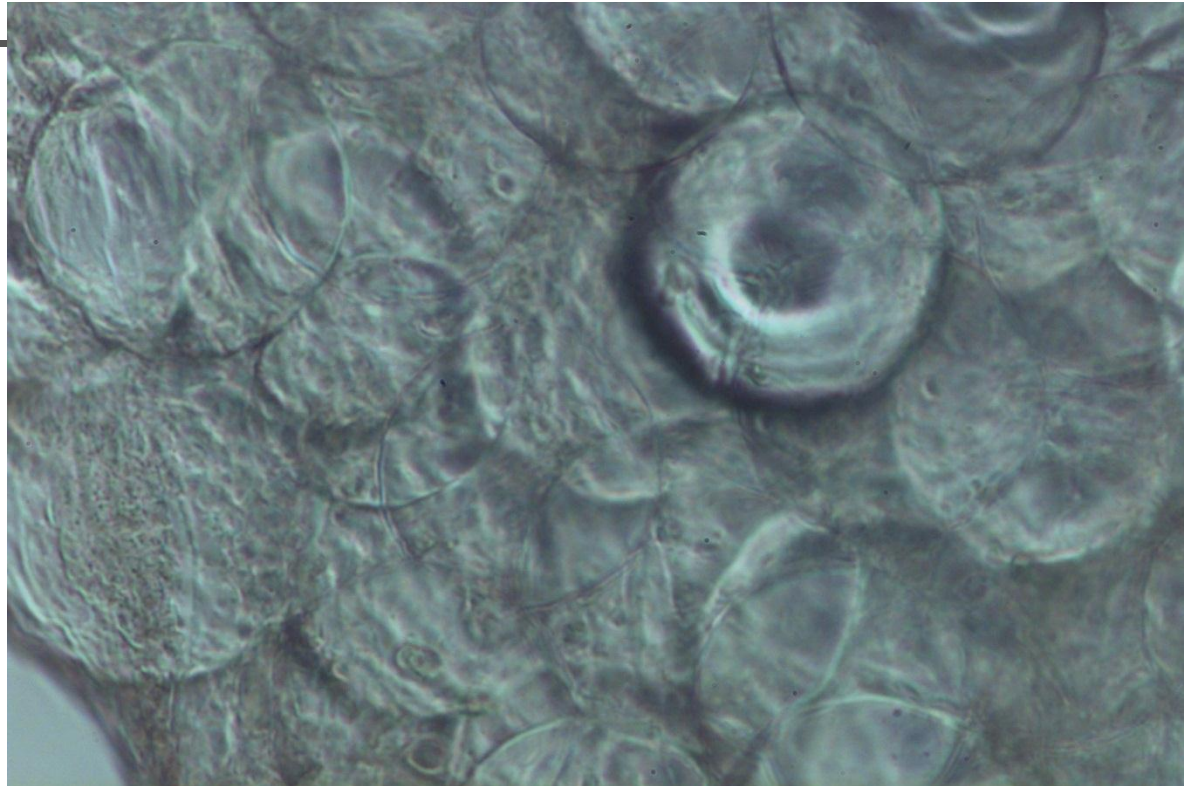


High Aspect Rotating Vessel
HARV

Osteoblast 3D Constructs

Phase Contrast

RWV
(7days)



- Human primary osteoblasts seeded onto collagen-coated Cytodex 3 beads and cultured for 7 days under static conditions followed by 7 days of culture in the RWV.



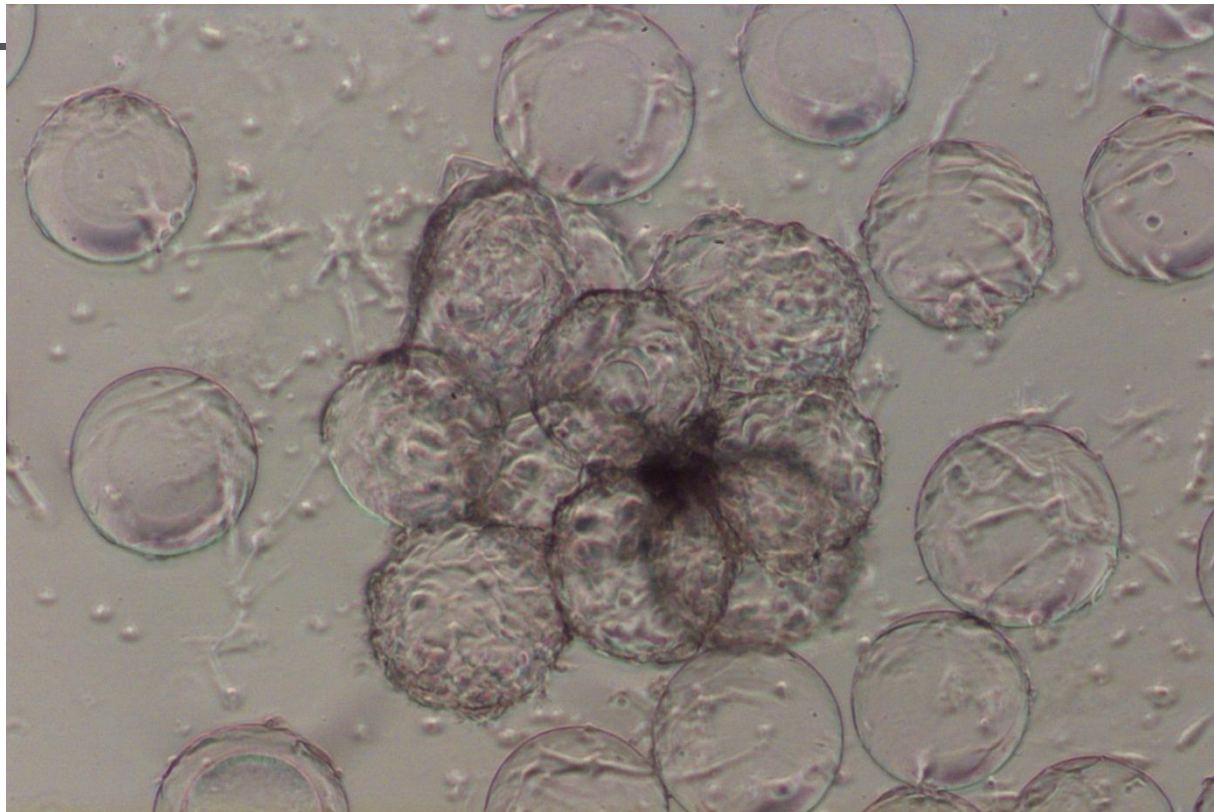
Results

- **Successful growth of human primary osteoclast precursor cells on collagen-coated beads under both static and RWV culture conditions.**
- **Osteoclast precursor cells were labeled with Cell Tracker™ dye in order to distinguish them from osteoblasts in preparation for co-culture experiments.**
- **Labeled osteoclast precursor cells were capable of differentiating into multi-nucleated osteoclast cells.**
- **Co-cultures of primary osteoblasts and osteoclasts were grown in the RWV without the use of Cytodex 3 carrier beads.**
- **Under specific rotation conditions, large 3D constructs up to 2 mm in diameter can be produced that contain both cell types.**
- **Osteoclasts, previously labeled with CellTracker™ dye, can be differentiated in the co-culture from osteoblasts.**
- **After a period of 7 days in the RWV, osteoclast cells appear to organize to the outer region of the 3-D construct.**
- **After a period of 14 days of culture in medium designed to promote mineralization, the constructs remains viable and takes on a smooth, spherical appearance.**
- **Determination of construct organization will require frozen sectioning followed by immuno-histochemical characterization due to the large size of the construct.**
- **Amelogenin was radioprotective in radiation exposed fibroblasts: Experiments with osteoblasts are now envisaged.**

Osteoclast 3D Constructs

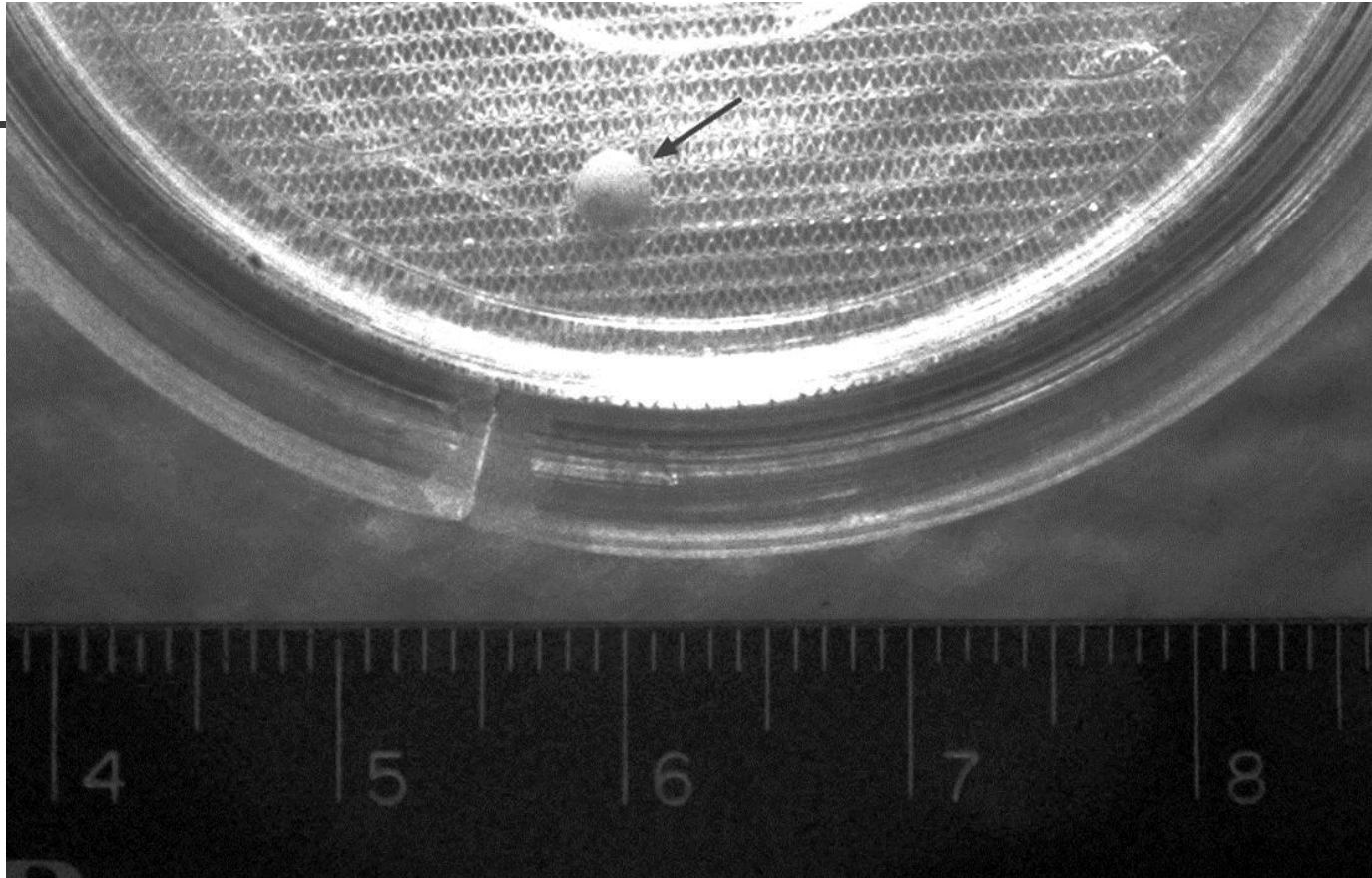
Phase Contrast

STATIC
(7days)



- Human primary osteoclasts seeded onto collagen-coated Cytodex 3 beads and cultured for 7 days under static conditions.

Osteoblast/Osteoclast Co-Culture



- Human Primary Osteoblast/Osteoclast 3D construct following 14 days of culture in the RWV under mineralization conditions. (Scale in cm)

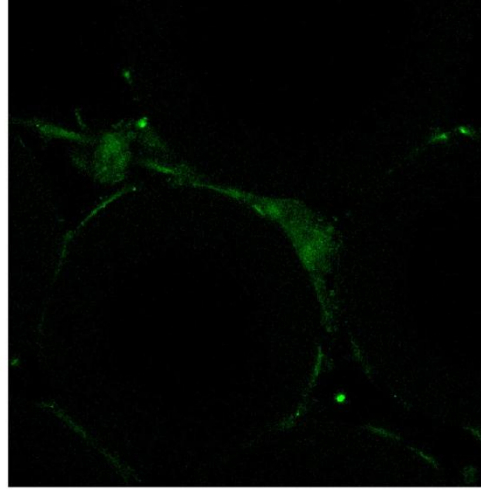
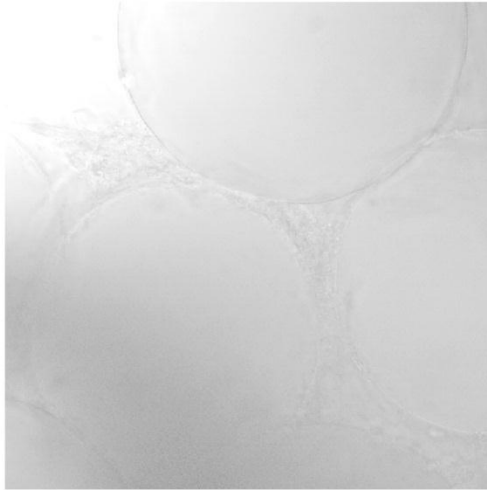
Osteoblast Differentiation Markers

Phase Contrast

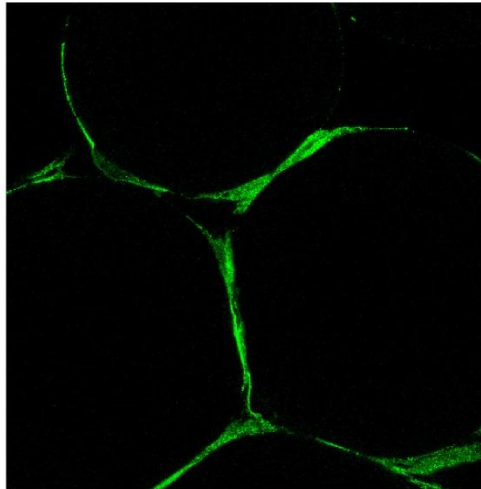
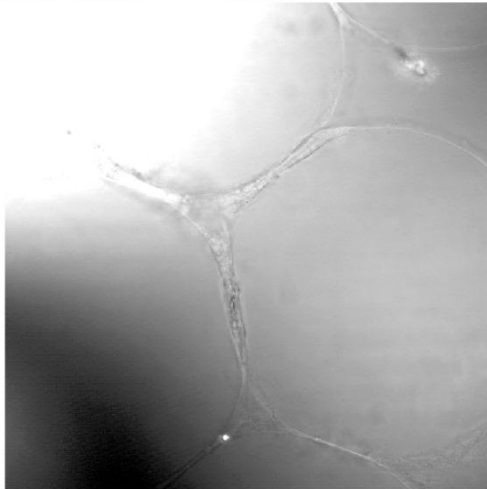
Immunofluorescent



STATIC
(7days)



RWV
(7days)



Osteocalcin
Staining
(early
differentiation
marker)

(0.3 micron optical
section using confocal
immunofluorescence
microscopy)

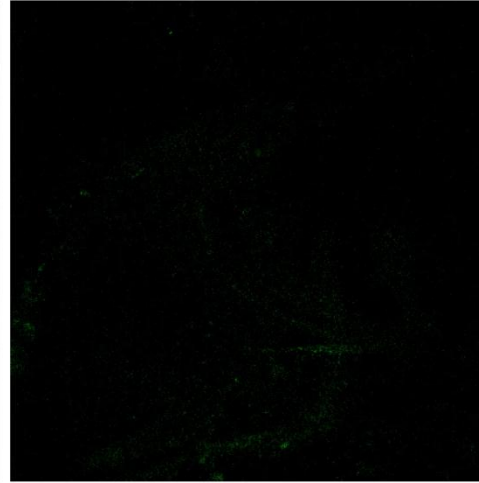
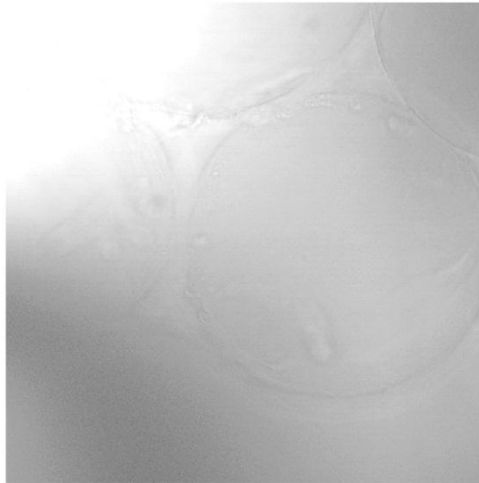
Osteoblast Differentiation Markers

Phase Contrast

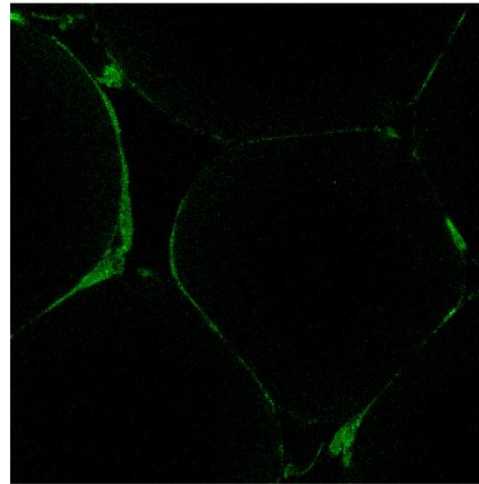
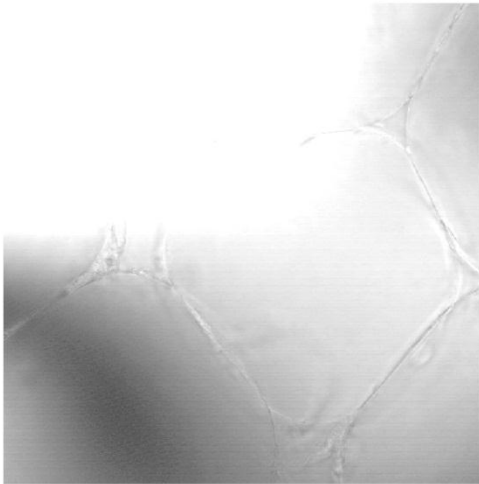
Immunofluorescent



STATIC
(7days)



RWV
(7days)

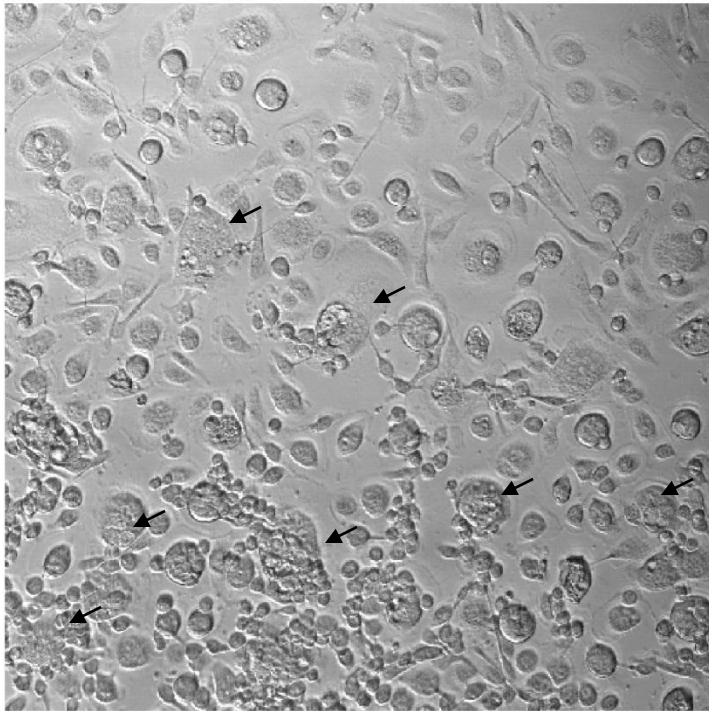


Alkaline
Phosphatase
Staining
(late
differentiation
marker)

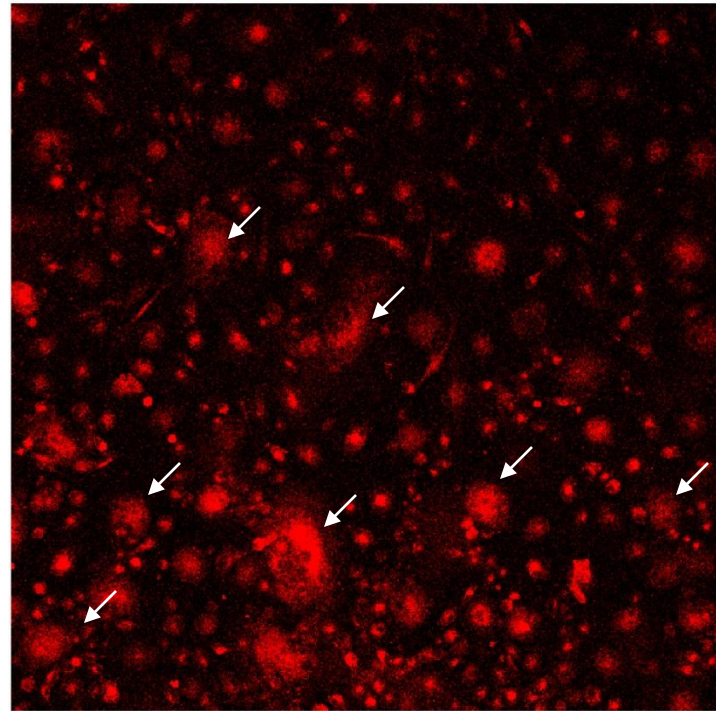
(0.3 micron optical
section using confocal
immunofluorescence
microscopy)

Osteoclast Differentiation

Phase



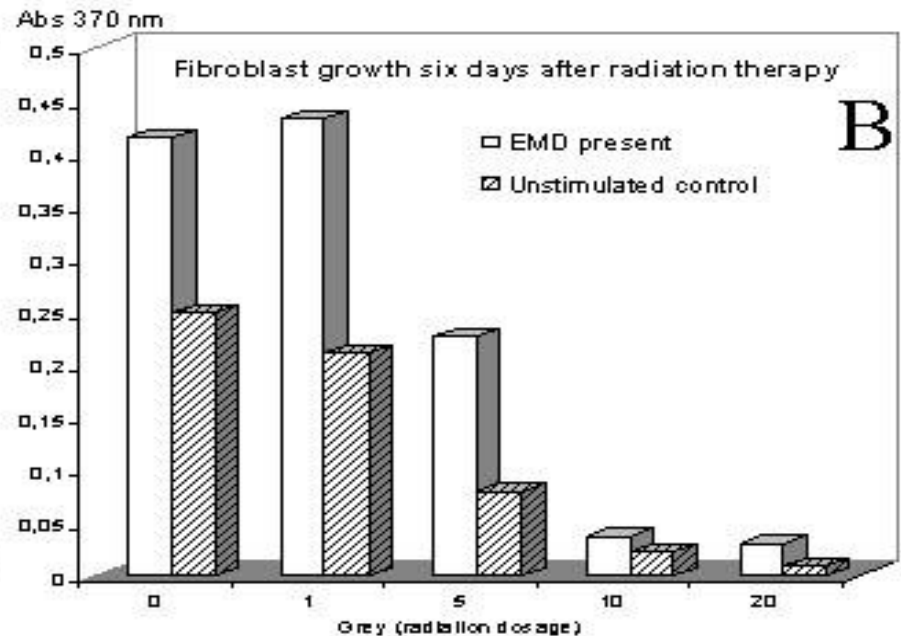
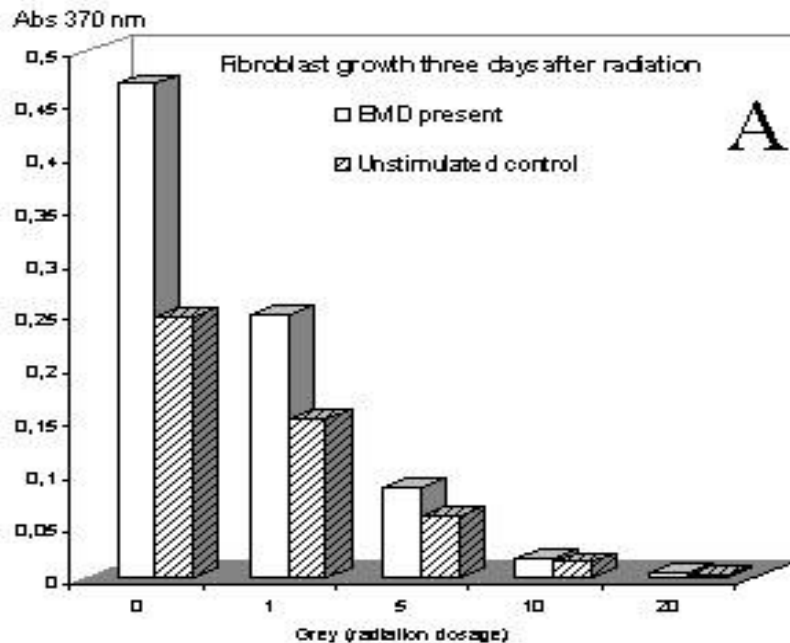
Cell Tracker™ Red



- Osteoclast precursor cells differentiated for 7 days in static culture induced to form osteoclasts (arrows, multi-nucleated cells)

Radiation damage and protection in fibroblasts as a prelude to osteoblasts

The ability of amelogenins to revitalize dermal fibroblast cells that have been exposed to ionising radiation has been tested in a pilot study. The cells received different gamma radiation dosages, followed by incubation with and without amelogenin present in the medium. Cell replication was significantly increased in the presence of amelogenin (Figure A and B). The results demonstrate that amelogenin proteins act as an epigenetic signal to stimulate cell proliferation of the subpopulation of cells in the culture that still is able to enter into the S phase after that the majority of cells have been irreversibly impaired by radiation damage.





Conclusions

The co-culture of osteoclast and osteoblast cells will provide new avenues in bone three-dimensional research. Biomarker discovery and risk factor identification can be investigated in this co-culture system.

Radiation works synergistically with some organ systems especially in relation to the inflammatory and immune response in organisms. Hence it will be important to assess the effects of microgravity combined with radiation exposure in bone tissue.

The effects of dose and cell function assessments such as apoptosis or cell death, bystander effects and bone cell proliferation will be assessed in osteoblasts and osteoclasts for a potential numerical model. This model will address both the synergism of radiation with microgravity and the relative kinetics of osteoblast function and osteoclastogenesis in microgravity