Preparation and Overview of Fish Scales Experiment

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Abstract: In space flight, bone resorption by osteoclasts has been observed inconsistently until now. The Japan Aerospace Exploration Agency (JAXA) carried out the Fish Scales experiment using the Measurement Experiment Unit (MEU) installed in the Cell Biology Experiment Facility (CBEF) on board Kibo. This experiment was designed to investigate the effect of microgravity on goldfish scales. Space shuttle STS-132 (ULF4), which carried the specimens, was launched to the International Space Station (ISS) on May 14, 2010. The experiment was started on May 16 and terminated on May 20, 2010. The MEU and CBEF environment control systems worked as planned. On May 26, 2010, the specimens cultured in Kibo were returned to Earth by space shuttle STS-132 and analyzed for their biological characteristics. In the Fish Scales experiment, a biological experiment including *in vitro* cell culture, temperature control was a key factor in the success of the experiment. In this report, experimental data regarding culture temperature in the CBEF and other temperature-maintaining systems during transportation between the ground and ISS are described, as well as the onboard operations by the ISS crew member. Judging by temperature data in the hardware under microgravity and in ground conditions, our experiment seems to have succeeded. The Fish Scales research team will analyze the cell activity, the osteoblastic and osteoclastic marker mRNA expression, and the cell morphology in the goldfish scales kept in microgravity. The results will be compared with ground controls and onboard 1G control scales to determine the effect of space on bone metabolism.
Key words: Temperature control, Regenerating scales, Osteoblasts, Osteoclasts, Bone metabolism under microgravity, Life sciences, International Space Station

I. Introduction

In space flight, bone resorption by osteoclasts has been observed inconsistently until now. To examine osteoclastic function under microgravity, a suitable *in vitro* assay system is required. Recently, the Fish Scales research team developed an *in vitro* assay system using goldfish scales [1,2] and then found that both osteoblasts and osteoclasts in the goldfish scales responded sensitively to hypergravity [3,4]. Therefore, our data will contribute to explaining the mechanism of bone loss, such as that experienced in space flight, as well as that of the gravity response to osteoblasts and osteoclasts.

The main objective of the Fish Scales experiment is to investigate the osteoblastic and osteoclastic responses to microgravity using goldfish scales. The teleost scale is a calcified tissue that contains osteoblasts, osteoclasts, and a bone matrix with two layers (the bony layer: a thin, well-calcified external layer; and the fibrillary layer: a thick, partially calcified layer). The bone matrix, which includes type I collagen, osteocalcin, and hydroxyapatite, is present in the scale as well as in mammalian bone. Furthermore, the teleost scale regenerates after being removed (see Figure 1). In a previous study, the Fish Scales research team reported that osteogenesis in regenerating scales was very similar to that in calvarial bone [5]. Osteoblastic activity in the regenerating scale was considerably higher than that in the normal scale [5]. In addition, the Fish Scales research team found that osteoblastic activity in regenerating scales significantly increased under 3G acceleration loading by vibration, while osteoclastic activity in the loaded regenerating scales significantly decreased [6]. Since fish scales can be kept in 4°C refrigeration before incubation for at least a week [7], the Fish Scales research team strongly believes that the regenerating scale is a good material for the

analysis of bone metabolism under different gravity environments.

In this report, the outline of the Fish Scales space experiment and an overview of the hardware used in Kibo are described. In the Fish Scales experiment, a biological experiment including *in vitro* cell culture, temperature control was a key factor in the success of the experiment. Thus, experimental data regarding culture temperature are also described.

II. Methods and Research Preparations

Timeline from selection to performance

The Fish Scales flight experiment was selected in 2008 as the second Japanese flight candidate. Experiment preparation for flight began after the selection, such as experiment requirement definition, hardware design, development, in addition to manufacturing and performance tests and a payload safety review. Operational product preparation, such as crew procedure, crew training, and the Multilateral Increment 23/24 symposium for science, was conducted in 2009 successfully. JAXA and the Fish Scales research team conducted specimen preparation rehearsal in Feb. 2010, which was also a success.

Hardware overview

The CBEF (Fig. 2 A) provides a controlled environment in terms of temperature, humidity, and CO_2 [8]. The CBEF has two sections: a microgravity section ("a" in Fig. 2 A) and an artificial gravity section of 0.1 g-2.0 g with a 35-cm-diameter turntable ("b" in Fig. 2 A).

The MEU (Fig. 2 B, outer size: W24 cm ×H11 cm ×D17 cm) is built in a box called the "Canister." A sample holding rack and temperature sensors are installed in the Canister, which is attached to the CBEF. A maximum of four Canisters can be installed on the turntable and six Canisters can be placed in the microgravity area of the CBEF.

The specimens are packed in the culture chambers for flight. Culture chambers are developed as part of the Cell Experiment Unit (CEU) then slightly modified for the Fish Scales experiment. The culture chamber (Fig. 2 C) consists of a gas permeable membrane and autoclavable components including Poly Chloro Tri Furuoro Ethylene (PCTFE), which enables recycling. The chambers are modified for the Fish Scales in order to pack scales more easily and to maintain a sterile condition. The Pre-fixation Kit (PFK, Pre-fixation apparatus, Fig. 2 D) and the Cell-fixation Kit (CFK, Fixation Cylinder, Fig. 2 E) are used to remove the cultured medium from the chambers; cell fixation in Kibo, as well as the CEU equipment, was developed by JAXA and provides sufficiently safe containment. The PFK was also used for RNAlater treatment in this experiment. To decrease pressure inside the chambers during fixation, the CFK has bags newly connected to the chambers.

Although the MEU has temperature sensors, the values of which were downlinked in real time, temperature loggers were placed closer to the culture chambers than the MEU sensor to obtain specimen temperature.

Method and protocol

Scales (regenerating) collected from goldfish under anesthesia were packed in culture chambers with a 96-well microtiter plate and the culture medium. They were assembled on a holder (Fig. 2 F) along with a temperature logger and launched at 4°C in a passive cool box, called a Double Cold Bag (DCB), with refrigerant. After arrival at the ISS, the chambers were incubated for 86 hours at 22°C under microgravity using the CBEF and compared with a 1G control in space. After the experiments, the scales were frozen at -95°C for cell activity analysis. Other scales for morphological analysis were preserved with a 4% paraformaldehyde phosphate buffer solution. For mRNA expression analysis, the scales were preserved with RNAlater, and then frozen. Cold stowage samples were installed in the MELFI (Minus Eighty degree Celsius Laboratory Freezer for ISS) refrigerated compartment or frozen compartment. During return on the space shuttle, samples were kept at 4°C in the DCB, or kept frozen in the active refrigerator, called GLACIER.

III. Results and Discussion

Table 1 shows the in-flight related activities of the Fish Scales experiment. The temperature of the loggers with samples showed the chambers were kept at 2.5- 4.0°C in the DCB in the launched space

shuttle and on the ISS before incubation. During incubation in the CBEF, all culture chambers installed in the microgravity section and 1G section of the CBEF were incubated at 21.9-22.0°C in the MEU. After chemical treatment, frozen samples were kept around -95°C and refrigerated samples were kept at 2°C in the MELFI. While returned in the space shuttle, the samples were kept at 4°C in the DCB, or kept frozen in GLACIER (-95°C). Returned samples were transported to Japan at the same temperature as during space transportation. Both space transportation and international transportation were conducted as planned.

During incubation in the CBEF, the turntable turned at 90 rounds per minute (rpm) +/- 1 rpm. The half diameter was 132 mm from the center of gravity to the 1G position of the MEU on the turntable. The target temperature was 22.0°C and the CBEF temperature setting was changed slightly from that of the ground depending on the downlinked temperature from the MEU sensors through the CBEF. Judging from the temperature data in the hardware under microgravity and in ground conditions, our experiment seems to have succeeded.

IV. Status of Data Analysis

The Fish Scales research team received frozen or fixed scales to examine any interaction between osteoblasts and osteoclasts in the scales on bone resorption under microgravity. The cell activity, the osteoblastic and osteoclastic marker mRNA expression, and the cell morphology in the goldfish scales kept in microgravity will be analyzed. The results will be compared with ground controls and onboard 1G control scales to determine the effect of space on bone metabolism.

V. Acknowledgments

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References

1) Azuma, K., et al.: Two osteoclastic markers expressed in multinucleate osteoclasts of goldfish scales. *Biochem. Biophys. Res. Commun.*, 362: 594-600 (2007)

2) Suzuki, N., et al.: Novel bromomelatonin derivatives suppress osteoclastic activity and increase osteoblastic activity: Implications for the treatment of bone diseases. *J. Pineal Res.*, 44: 326-334 (2008)

3) Suzuki, N., et al.: Effect of vibration on osteoblastic and osteoclastic activities: Analysis of bone metabolism using goldfish scale as a model for bone. *Adv. Space Res.*, 40:1711-1722 (2007)

4) Suzuki, N., et al.: Scale osteoblasts and osteoclasts sensitively respond to low-gravity loading by centrifuge. *Biol. Sci. Space*, 22: 3-7 (2008)

5) Yoshikubo, H., et al.: Osteoblastic activity and estrogenic response in the regenerating scale of goldfish, a good model of osteogenesis. *Life Sci.*, 76: 2699-2709 (2005)

6) Suzuki, N., et al.: Response of osteoblasts and osteoclasts in regenerating scales to gravity loading. *Biol. Sci. Space*, 23: 211-217 (2009)

7) Suzuki, N., et al.: Fish Scales study for space biology: Application of scale culture method in the space environment. *Space Utilization Res.*, 25: 166-169 (2009) (in Japanese)

8) Ishioka, N., et al.: Development and verification of hardware for life science experiments in the Japanese Experiment Module "Kibo" on the International Space Station. *J. Gravit. Physiol.* Mar., 11:81-91 (2004)

Table 1 Fish Scales Experiment Sample (scales) Related Activities (All dates in 2010)

Time (Greenwich Mean Time)	**Activities and Description**
May 10, 9:00	Finish packing regenerating scales in culture chambers in Japan
May 10, 20:00	Finish packing culture chambers for transportation from Japan to Kennedy Space Center
May 11, 20:00	Arrival of culture chambers at Kennedy Space Center
May 12, 18:00	Turn over cold stowage sample to NASA in DCB
May 14, 18:24	STS-132 (ULF4) launch
May 16, 19:00	Start experiment, transfer Fish Scales samples from shuttle DCB to CBEF (for culture) and MELFI (for launch control)
May 20, 9:00	Terminate incubation and chemical treatment, fix specimen with chemical solutions using PFK and CFK, transfer Fish Scales samples to MELFI
May 26, 16:30	Receipt of samples at Kennedy Space Center

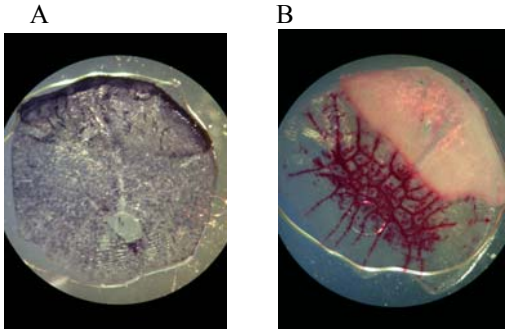

Fig. 1 Fish Scales images and captions: ALP staining for osteoblasts (A); TRAP staining for osteoclasts (B) in regenerating scales of goldfish (ALP: alkaline phosphatase; TRAP: tartrate-resistant acid phosphatase)

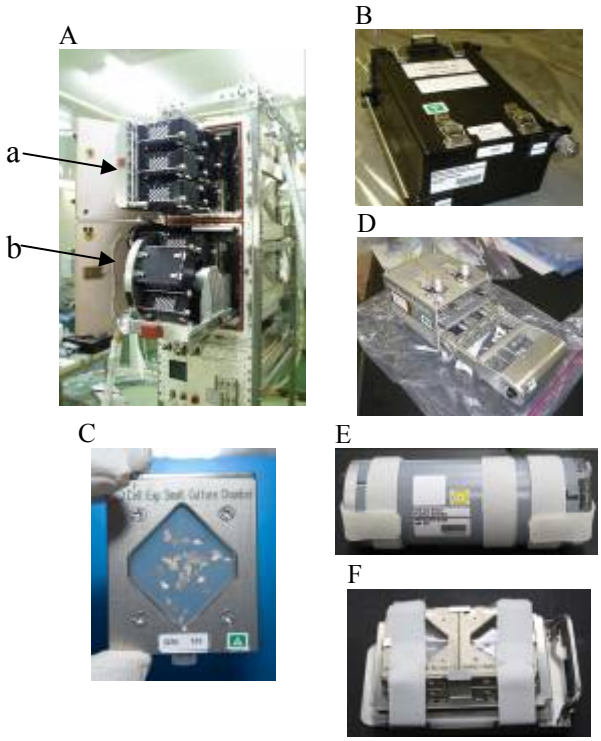

Fig. 2 Fish Scales Experiment hardware: CBEF (A) a microgravity section; “a”, an artificial gravity section; “b”; MEU (B); Fish Scales culture chamber with scales (C); Pre-fixation Kit (E); Cell-fixation Kit (D); Sample holder assembly (F)