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**Technical report**

# A novel femur window chamber for *in vivo* studies of bone microcirculation

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***Background:*** Due to technical difficulties of *in vivo* observation of blood flow and microvessels in bone, no study has been done concerning the role of blood flow in bone remodeling.
***Objective:*** To develop a new window chamber for microscopic observation of the microcirculation in living bone, and to examine the utility of the chamber using rat femur in health and diseases.
***Methods:*** A stainless chamber (19 mm in diameter and 5 mm in height) with a circular window (7.5 mm in diameter) for microscopic observation was developed. The chamber was put on rat femur which was exposed for direct observation of the microvasculature. Intravital observation was made of bone blood flow and microvessels, using fluorescence videomicroscopy and confocal laser microscopy. The utility of the chamber was examined based on images of microcirculation (normal and abnormal) in the femur bone.
***Results and conclusions:*** Images of rat femur microvasculature were enhanced in the quality by use of the femur window chamber. The new chamber provides a powerful tool for *in vivo* studies of the bone microcirculation in health and diseases.

***Keywords:*** Bone, confocal laser microscopy, femur bone, fluorescence videomicroscopy, leukocyte adhesion, microcirculation, window chamber.

The dynamics of bone remodeling may be determined by complex sequential interaction between bone cells and microcirculation [1]. Blood flow in the bone may be intimately related to bone remodeling, bone formation and resorption [2-7]. However, no study has been done on the role of blood flow in bone remodeling; mainly due to technical difficulties of *in vivo* observation of bone blood flow and microvessels.

A number of window chambers for intravital observation of microcirculation have been developed in various organs and tissues, including cranial windows [8-10], dorsal skin chambers [11-13] and iris devices [14, 15]. However, there are very few bone window chambers available for direct microscopic observation except for the Hansen-Algenstaedt chamber [16].

In this study, we developed a new window chamber for intravital observation of the microcirculation in femur by modifying the femur window previously used by Hansen-Algenstaedt et al. [10]. The new window chamber could prevent scatter of light and enhance focusing on the object. With the use of this window chamber, we could obtain good quality microscopic images of the bone microcirculation.

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## Materials and Methods

### *Design of a femur chamber*

We developed a stainless steel chamber for rat femur as shown in **Fig. 1.** The size was designed based on the structure of rat femur. It was approximately 19 mm in diameter and 5 mm in height. The chamber was also designed to have a closed and circular window (approximately 7.5 mm in diameter and 5 mm in height) for microscopic observation. The chamber was positioned on the study area of the femur. Prior to placement of the chamber, the femur was exposed around the study area for direct observation of bone blood flow and the microvasculature.

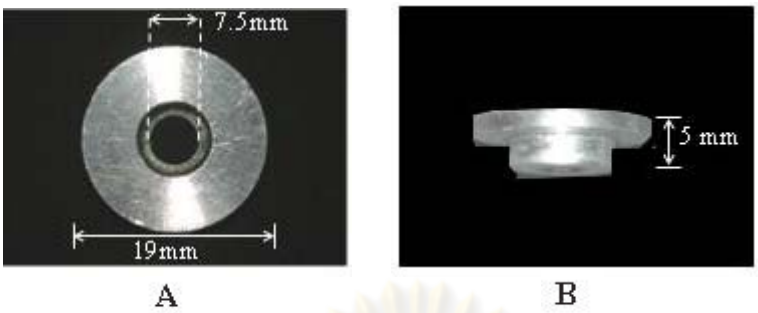



**Fig. 1** A newly developed window chamber for rat femur.

***Performance test of the window chamber***

We examined the performance of our newly developed window chamber in the rat femur under a fluorescence videomicroscopic system and a confocal laser microscopic system. The performance of the window chamber was evaluated based on the quality of images of bone microcirculation obtained with and without the chamber.

***Animal preparation***

Rats were anesthetized with sodium pentobarbital (45 mg/kg bw, i.p.). After tracheotomy, a polyethylene catheter was inserted into the left external jugular vein for injection of fluorescent tracers.

A longitudinal skin incision was made approaching the femur laterally. The femur was carefully exposed by blunt dissection between the flexors and extensors muscles to visualized bone vasculature. Our femur chamber was positioned on the study area for an enhanced deeper focus (**Fig. 2**).

***Microscopic obsveration***

After anesthesia, fluorescent tracers were injected to visualize the microcirculation in the femur. The bone microcirculation was observed using an epi-illumination fluorescence videomicroscope (Optiphot-2, Nikon, Japan) with a 50 W mercury lamp. The video images were achieved through a silicon intensified target television camera (Hamamutsu Photonics, Japan, USA) [11-12], which was projected on a video monitor (GM-1411 QM, Sony, Japan) using 20X objective lens (CF Plan Fluor, Nikon, Japan). The selected area was then recorded in real-time by a videotape recorder (SLV-X311, Sony, Japan) connected to a video timer (VTG-55, FOR-A, Japan) throughout the experimental period.

***Confocal microscopic observation***

The bone microvasculature was observed under a confocal laser microscope (EZ-C1, Nikon, Japan). An objective lens of 10X and a laser unit (488 argon)

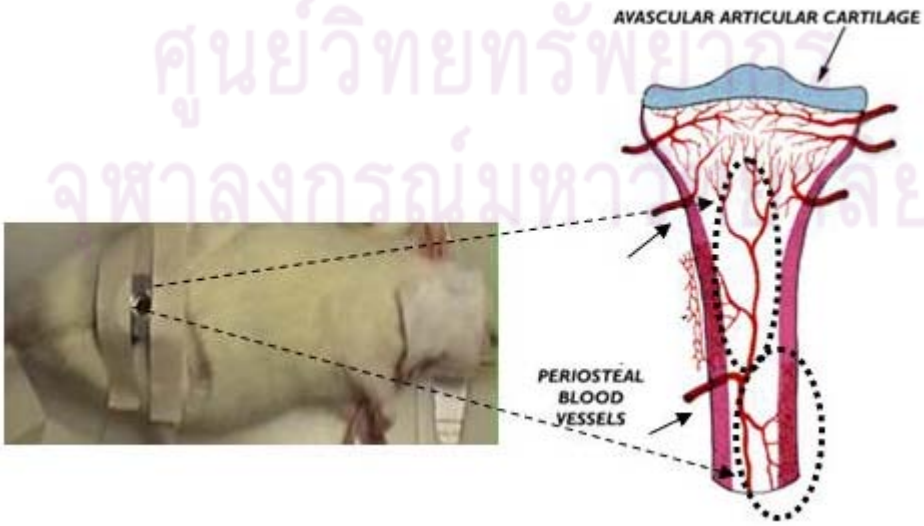



**Fig. 2** The location of the femur chamber is shown. The bone microcirculation, feeding from arteries that penetrated through priosteal membrane, can be observed via this chamber.

were used. Fluorescein isothiocyanate (FITC)-dextran (50 ml of 0.5 % FITC-dextran, Sigma Chemical, USA) was injected intravenously to visualize the intralumen of microvessels.

***Pathophysiological application***

As an example of bone pathophysiology, we studied microvascular disorders, such as increased interaction between leukocytes and endothelial cells, in ovariectomized rats [17].

## Results

**Figure 3 (A, B)** show examples of video images of bone microcirculation in an era of the femur with (**B**) and without (**A**) use of the window chamber. We note that the use of femur chamber provided better quality images. Apparently, leukocytes could not be identified without the chamber, but with use of the chamber, leukocytes and endothelial cells interaction could be observed in femur postcapillary venules (diameter =15-25 mm).

**Figure 4** shows a confocal microscopic image of femur microvessels that was taken with use of the femur window chamber. By using the femur window chamber, we could observe the femur bone microcirculation under a confocal laser microscopic system.

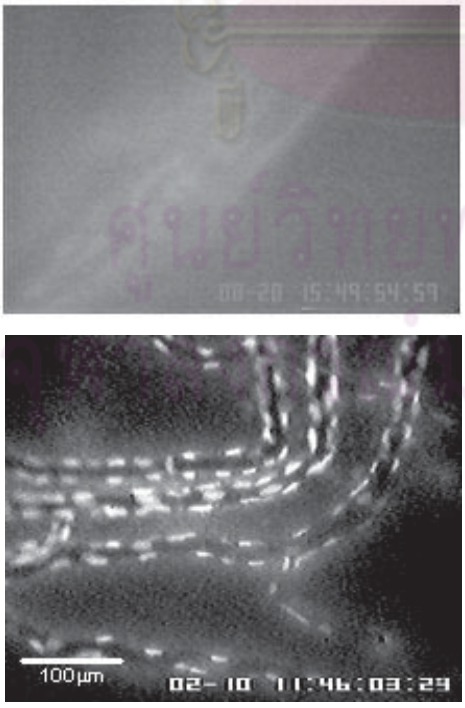
**Figure 5** shows an example of fluorescence video image. Apparently, a number of leukocyte adhered to the venular wall in post-capillaries of ovareictomized rats.

## Discussion

In the present study, we have made a femur window chamber by modifying the femur window previously used by Hansen-Algenstaedt et al. [16]. The chamber could improve the quality of images obtained under a fluorescence microscope as well as confocal laser microscope. In fact, the chamber helped to prevent the scatter of light, so that the light was condensed and passed through the window of the chamber, resulting in enhancement of the focusing on the object.

With use of this femur chamber, the leukocyte-endothelial cell interaction in sham and ovariectomized rats could be evaluated using intravital fluorescence videomicroscopy. Moreover, their microvascular network of bone capillaries was demonstrated clearly by using confocal laser microscopy.

In conclusion, this novel femur window chamber provides a powerful tool for *in vivo* studies of bone microcirculation. In particular, it will be useful for investigation of the *in vivo* model of estrogen depletion induced pathogenesis in the future.




**A** Without chamber x20

**B**. With chamber x20

**Fig. 3** Examples of video images of the microcirculation in an era of femur bone with (**B**) and without (**A**) use of the window chamber.

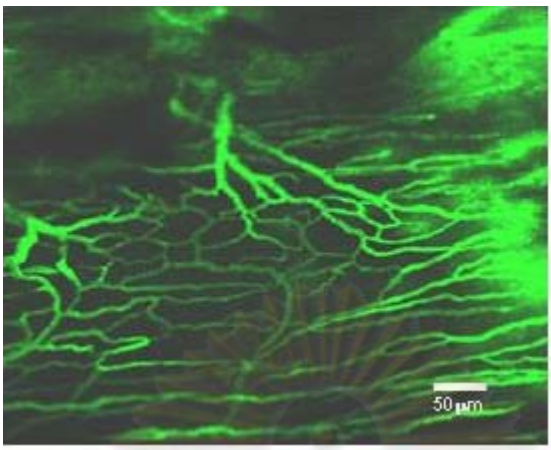



**Fig. 4** A confocal microscopic image of the femur bone microcirculation observed with use of the femur window chamber. After penetration through priosteal membrane, the microvascular network of capillaries branched were performed and run along the bone.

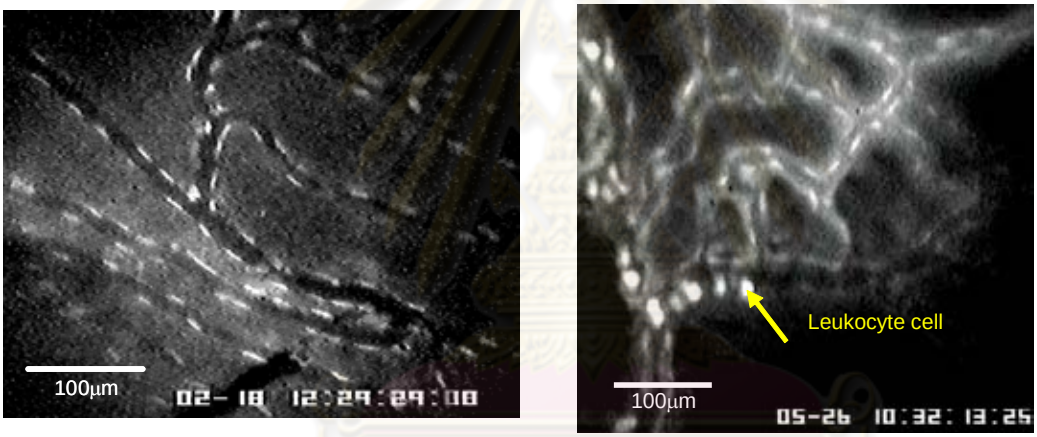



**Fig. 5** Fluorescence video images obtained in normal (sham) (**a**) and ovariectomized rats (**b**) (bar: 100 μm). In (**b**), there appeared turtosity microvascular network and leukocyte adhesion.

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