INTRODUCTION
Osteonecrosis of the femoral head (ONFH), a disease of unknown pathogenesis, usually involves subchondral bone. Autologous block bone grafts taken from the femoral head were reported to be revascularized, incorporated and remodeled completely 18 months after surgery (1). Unlike the grafted block bones, the necrotic lesions in ONFH seem to be regenerated poorly. Reparative process is limited at the margin and does not progress to the inside of a necrotic lesion (2). The temperature of the subchondral bone of the femoral head was found to increase by a maximum of 2.5 °C in human hip joint in vitro in simulated walking (3). More increase in the temperature is expected in the necrotic bone in ONFH because heat dissipation by the blood flow does not occur. We postulated two possible harmful effects of high temperature on the regeneration of the necrotic area. The vascular ingrowth might be inhibited by the high temperature itself and/or by the necrotic bone substance denatured by the high temperature. We evaluated the effects of high temperature and denatured necrotic bone substance on the proliferation of human endothelial cells.

METHODS
Production of necrotic and living bone extracts: Using endothelial cell growth media-2 (EGM-2) (BioWhittaker, Inc., Walkersville, MD, USA), necrotic and living bone extracts were prepared from the femoral heads of four ONFH patients who underwent THA. The bone tissues were ground with freezer-mill aseptically. The ground bone particles were suspended at a concentration of 0.1g/ml in EGM-2. The bone particles/EGM-2 suspension was agitated for 24 hours at 37 °C and centrifuged at 1500g for 30 min. The supernatant was filtered with 0.2 μm filter membrane (Nalgene, Rochester, USA) to collect the bone extracts.

Cell culture: Human umbilical vein endothelial cells (HUVECs) (BioWhittaker, Inc., Walkersville, MD, USA) were initially plated in 96-well plates at a concentration of 1x10^3 cells/well and cultured in EGM-2. To evaluate the effect of hyperthermia, the cells were cultured in EGM-2 at temperatures of 40, 41, and 42 °C from the second day of culture, whereas the extracts prepared from the normal living bone had stimulatory effects. These data support that local subchondral hyperthermia in the necrotic area is a possible cause of poor regeneration of the necrotic area in ONFH.

RESULTS
The number of viable cells decreased markedly in hyperthermic conditions of 41 °C to 42 °C compared to control condition of 37 °C (p<0.05) (Fig. 1). The addition of 0.02 g/ml living bone extracts induced a significant increase in the number of viable cells at days 6 and 8 (p<0.05). Necrotic bone extracts did not induce such a significant increase. At day 8, the number of cells treated with necrotic bone extracts decreased significantly (Fig. 2).

DISCUSSION
There is a hypothesis for the pathogenesis of ONFH based on the inhibition of angiogenesis (4). The reason for an improper repair process of the necrotic subchondral bone in ONFH has not been elucidated clearly. In this in vitro study, it was found that hyperthermia had definitely inhibitory effect on the proliferation of human endothelial cells. Bone extracts prepared from the osteonecrotic portion of human femoral head inhibited the proliferation of human endothelial cells, whereas the extracts prepared from the normal living bone had stimulatory effects. These data support that local subchondral hyperthermia in the necrotic area is a possible cause of poor regeneration of the necrotic area in ONFH.

REFERENCES