Does heat developed during robot-assisted THA affect the osteoblastic activity of cells on cutting surface?

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Introduction: Total hip arthroplasty (THA) is one of the most successful surgeries in history of medicine. To add reliable surgical precision to the surgery, surgical robots for arthroplasty have been developed and tried. The simple concept for surgical robot is that it mills the bone surface exactly as planned before the operation. Theoretically, this exact performance can bring excellent alignment optomal fixation in every cases. However, there are several well-known obstacles. Among them, the thermal effect related to the high-speed burr attracted the author's attention. Although the robot has its own irrigating system, the heat developed during milling a bone can cause a burn to the bone and adjacent tissues. Although the exact temperature has not been published, we believe it is sufficient to destroy the cells and proteins in the remained bone matrix. If there is any thermal damage, biologic healing including bone in-growth cannot successfully achieved. To date, there has been only one clinical report that has claimed that successful bone in-growth on the milled surface has been observed. But as far we know, there has been no biologic research about this topic. Our basic question for this study was simple; does the heat affect the cellular activity of the cells obtained on the milled surface during robot-assisted THA?

Materials & Methods: After the informed consents were obtained from the patients and their families, we used 21 femoral heads obtained during consecutive robot-assisted THA using ROBODOC (Curexo technology, Davis CA, USA) in the same institutes. The diagnosis leading to the operation was osteonecrosis of the femoral head (ONFH) in all cases. After the operation, the femoral heads were stored in deep-freezer (-80°C). The average time for storage was 5 months (3-7 months). Samples from the bone marrow were obtained in two different places in each femoral head. After thawing, we obtained 10cc of the trabecular bone throughout the milled surface with a curette. These samples were enrolled as a study group. In the same femoral head, the matched control trabecular bone was obtained from at least 1cm apart from the milled surface. To avoid the sampling error, the necrotized area was not included in all samples. The trabeular bones were put in 30㎖ Phosphate Buffered Saline (PBS) up to 5㎖. Then, PBS was suctioned and filled up with trypsin. Primary cells were trypsinized for 30 min by orbital shaker in incubator at 37℃. Cells was isolated by cell strainer (40㎛, BD falcon, REF 352340), then washed by DMEM without serum. Cells were seeded into dish (6 well dish for MTT assay, 92006, 60 x 15mm dish for digital picture and osteocalcin and alkaline phosphatase, 93060, TCCs) for each experiment. Then, they continued to culture in the CO2 incubator at 37℃.

The cell pictures were taken with Olympus optical microscope ckx41 of digital camera for observing the changes of cell morphology. After 12 hr for cell stability, time was set 0hr. MSCs have taken picture from 5 different time conditions, 0hr, 4hr, 12hr, 24hr, 48hr. Cells were seeded in 12 well(SPL, Tissue Culture Testplate) by different time(0hr, 4hr, 12hr, 24hr, 48hr). A total of 100㎛ of MTT solution (Sigma, St Louis, MO, USA) was added to each well. After incubation, MTT was aspirated and 100㎛ per well of DMSO (Dimethyl sulfoxide) was added to each well. Subsequently, ELISA Reader read the optical densities of plates at 540nm. OC assay was made with the kit (Invitrogen; KAQ1381) at 24hr. Bound enzyme-labeled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB ready for use) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiter plate is then read at 450nm wavelength. Alkaline phosphatase activity was determined using a kit for ALP (Sigma. APF). Culture medium was collected

at 24hr, same as osteocalcin condition. Incubate samples at 65 °C for 15-30 minutes. Negative control was DMEM without serum.The fluorometer was set to 355 nm excitation and 460 nm emission.

Results: There were no differences in morphology of the cells in both groups in each time period (0, 4, 12, 24 and 48 hours after 12 hours of initial stabilization). MTT assay reveals that the viability and proliferation of the cells in both groups were not different in the same time periods. Total alkaline phosphatase activity of the cells from milled surface was 4.5% lower than that of non-milled surface. Total OC activity from milled surface was 6.8% higher. However, paired analysis revealed that there were no differences in either activity of the cells from both groups.

Conclusion: With this study, we found that there was no difference in viability, proliferation and osteoblastic activity between cells on the milled and un-milled surface. This suggests that the heat generated during high-speed milling does not alter the osteoblastic activity of the cells as long as sufficient irrigation was provided. Thus, it will not cause negative effect on the clinical results of the robot-assisted total hip arthroplasty.