—Original—

Effects of Endoprosthesis Head Material on Acetabular Cartilage Metabolism: An Animal Study Using Crossbred Pigs

Shuhei Matsui[1], Tokifumi Majima[1], Katsumi Mominoki[2], Haruko Hirayama[2], Yasushi Oshima[1], Kenji Takahashi[1] and Shinro Takai[1]

[1]Department of Orthopedic Surgery, Nippon Medical School, Tokyo, Japan
[2]Advanced Science Research Center, Okayama University, Okayama, Japan

Background: Hip endoprosthesis is one option for the treatment of displaced femoral neck fractures and avascular necrosis of the femoral head. Few reports are available describing acetabular cartilage metabolism after endoprosthesis surgery of the hip. The purpose of this study was to compare the biological effects on cartilage between cobalt-chrome (Co-Cr) and alumina ceramic heads wherein the cartilage articulates directly.

Methods: We used the acetabular cartilage from six hips of three immature crossbred pigs to examine the effects on cytokines, the amount of hyaluronic acid (HA), and cartilage mRNA expression of ceramic head and Co-Cr head endoprosthesis. Mechanical loading of materials of Co-Cr and ceramic heads was performed on the acetabular cartilage in culture media as an organ culture model. Thereafter, protein levels of cytokines (MMP-1, 3, TNF-alpha (α), Interleukin (IL)-1 alpha (α), and IL-1 beta (β)) and the amount of HA were measured from the culture media. Cartilage RNA extraction was performed, and quantitative reverse transcriptase-polymerase chain reaction was performed with primer sets for type I, II, and III collagens; aggrecan; MMP-1, 3, 13; TNF-α; and IL-1 α, IL-1 β.

Results: Protein level of IL-1 β and amount of HA in the Co-Cr group were significantly higher than those of the Ceramic group. Type II collagen mRNA expression in the Ceramic group was significantly higher than in the Co-Cr group. IL-1 β mRNA expression was significantly higher in the Co-Cr group than in the Ceramic group.

Conclusions: The present study showed that ceramic bipolar produces smaller adverse effects on cartilage cells compared to Co-Cr bipolar. These results could have significant implications for implant usage not only in hip joints, but also in other joints, including the shoulder, talus and radial head.

(J Nippon Med Sch 2018; 85: 309–314)

Key words: hip end-prosthesis, alumina ceramic, cobalt-chrome, acetabular cartilage metabolism, cytokine

Introduction

Hip endoprosthesis is one option often employed for the treatment of displaced fractures of the femoral neck and avascular necrosis of the femoral head in Asian countries[1,2]. A Cobalt-Chrome (Co-Cr) head is used quite often for the hip endoprosthesis. Pain or discomfort is frequently reported after this surgery with Co-Cr[3]. This may be caused by the effect of metal on cartilage metabolism. There are some reports that long-term results of hip endoprosthesis implantation using metal, including Co-Cr, have not been satisfactory. For example, Posada et al[4] reported that prolonged exposure to Co-Cr wear debris released from a resurfacing hip implant induces lymphocyte proliferation, suggesting that activation of resting lymphocytes may have occurred. Shah et al[5] reported that Co-Cr exposure affects osteoblast function and impairs the mineralization of the prosthesis surface. Furthermore, acetabular erosion was reported to occur due

Correspondence to Shuhei Matsui, MD, Department of Orthopedic Surgery, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8603, Japan
E-mail: s8086@nms.ac.jp
Journal Website (http://www2.nms.ac.jp/jnms/)

to the metal outer head postoperatively[6–9].

It has been reported that alumina and zirconia ceramic is bioinert. Ishida et al developed an original alumina ceramic bipolar hip prosthesis and reported that the surface of the ceramic head was less rough than that of a metal head, and that the change of the shape of the ceramic head over time was smaller inside the human body[1]. In past reports, there have been some studies describing the long-term results of Co-Cr versus ceramic heads in total hip arthroplasty[10–12]. However, there have been few reports related to acetabular cartilage metabolism after hip endoprosthesis replacement.

The purpose of our present study was to compare the biological effects on cartilage between Co-Cr and alumina ceramic heads in direct articulation with cartilage. Specifically, we compared the effects on acetabular cartilage metabolism between the alumina ceramic head endoprosthesis and that of the Co-Cr head in an organ culture model, concerning the protein level of inflammatory cytokines (Matrix metalloproteinase (MMP)-1, MMP-3, Tumor necrosis factor (TNF)-α, Interleukin (IL)-1 alpha (α), and IL-1 beta (β)), amount of hyaluronic acid (HA) in culture media, and cartilage mRNA expression (type I collagen, type II collagen, type III collagen, aggrecan, MMP-1, MMP-3, MMP-13, TNF-α, IL-1 α, and IL-1 β). The hypothesis of this study was that ceramic heads have a smaller adverse effect on cartilage metabolism than Co-Cr. Moreover, if confirmed, this would have significant implications for implant usage, not only in hip joints, but also in other joints including the shoulder, talus and radial head.

Materials and Methods

Animals

For the present study, we used three healthy immature crossbred pigs (Duroc and Landrace × Yorkshire). The average weight of the animals was 36+/−3.6 kg. The animals were killed using an overdose intravenous injection of 1 mEq/mL potassium chloride solution under deep anesthesia with 3% isoflurane inhalation. Just after death, the acetabula of both hip joints were retrieved from each pig and all soft tissues were removed. Thus, the total number of the acetabula was six. The average acetabulum diameter was 26.3+/−0.6 mm. This animal experiment was performed at Okayama University, according to the animal use protocol reviewed by the Animal Use and Care Committee, Okayama University (approval number: OKU 2013436).

Loading Protocols

Mechanical loading was performed two hours after death. A compression and rotational stress was applied to the acetabula using an Instron MTS testing machine (MTS systems, MN, USA). Each acetabulum was fixed to the mount of the MTS machine using bone cement. We used 26 mm diameter femoral heads of Co-Cr (Nakashima Medical, Okayama, Japan) and alumina ceramic (CeramTec, Plochingen, Germany). The load cell was connected to the femoral head and was set up with the acetabulum in the culture medium. The culture medium included Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific Inc. Waltham, MA, USA) with 10% Fetal Bovine Serum (FBS) (Biowest, Nuaillé, France), 1% Penicillin, and ascorbic acid. The culture media was placed in a water bath of 37 degrees Celsius in order to simulate body temperature during the treatment (**Fig. 1**). Ten thousand cycles of compression and rotation to cartilage was applied at 1 Hz. The axial compression was controlled by force, and rotation was controlled by the rotating angle (1,500 N to 150 N of compression and 12 degrees of rotation) (**Fig. 2**). The applied axial load of 1,500 N corresponds to 5 times the pig's body weight. It is assumed that 1,500 N is the maximum load during trotting. The right acetabulum was loaded with a Co-Cr head, and the left acetabulum was loaded with a ceramic head for each pig, respectively.

Analysis of Protein Levels

The culture medium was retrieved from the water bath and stored frozen until analysis. Analysis of protein levels of inflammatory-related cytokines (MMP-1, MMP-3, TNF-α, IL-1 α, and IL-1 β) was outsourced to a clinical laboratory testing company (SRL, Tokyo, Japan). We measured HA concentrations in culture media using a hyaluronan assay kit from Seikagaku Corporation (Tokyo, Japan). The samples of acetabulum after the mechanical loading were retrieved and stored at −70 degrees Celsius until RNA analysis.

RNA Isolation and Relative Quantitative RT-PCR

Cartilage RNA extraction was performed according to a previously reported method[13]. Total RNA was quantified fluorometrically using the SYBR® Green Reagent (Molecular Probes; Eugene, OR, USA). Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed with specific primer sets for type I collagen, type II collagen, type III collagen, aggrecan, MMP-1, MMP-3, MMP-13, TNF-α, IL-1 α, and IL-1 β.

The acetabular cartilage was shaved off with a graver. Thereafter, the cartilage was powdered according to pre-

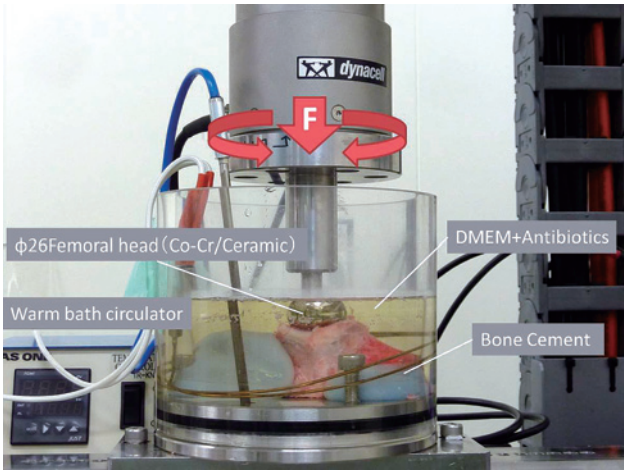

Fig. 1 Photograph of the apparatus for loading treatment

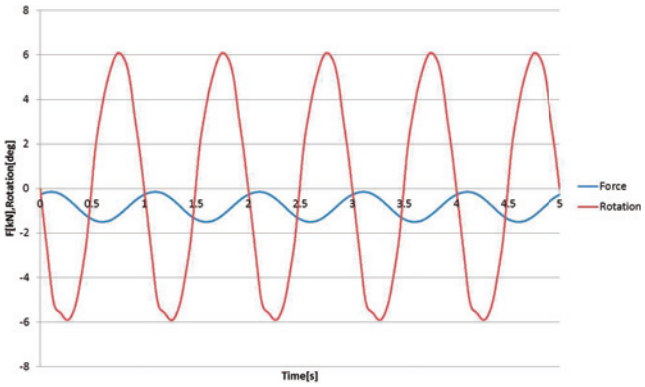

Fig. 2 Loading protocols

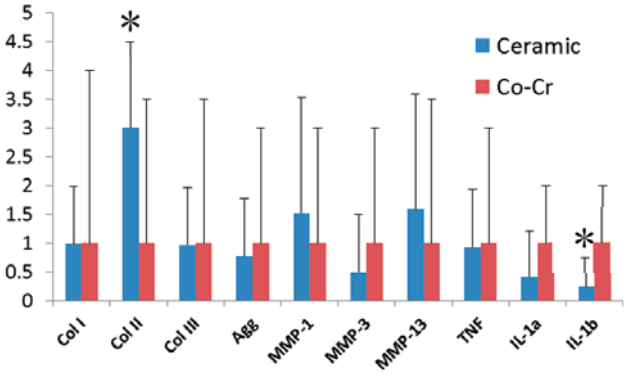

Fig. 3 Quantitative mRNA expression of each gene. mRNA expression of the Ceramic group was written as a comparison value based on the value of the Co-Cr group.
*: $p<0.05$

viously reported methods[14–16]. Total RNA of the cartilage was extracted with TRIzol and was refined by a buffer solution and centrifuge. After the extraction, 2 µg of total RNA was reverse transcribed into cDNA using the High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Waltham, MA). Aliquots (2.0 µL of 20 µL total volume) of the resulting cDNA were amplified in a total volume of 20 µL containing Taqman Fast Advanced Master Mix and each primer by 40 cycles with a genetic amplification machine (Thermo Fisher Scientific, Waltham, MA). Experimental conditions were determined to be in the linear range for both the PCR amplification and the image analysis system. Integrated density values for the genes in question were normalized using the corresponding GAPDH value for a quantitative assessment. The information for each primer set is detailed in **Table 1**.

Statistical Analysis

Statistical analysis was performed using STATWING (www.statwing.com). Statistical comparisons were carried out using Welch's t-tests with a significant threshold equal to $p<0.05$. Data are represented as means +/− standard deviation (SD).

Results

The protein level from culture media of the Co-Cr group in MMP-1, MMP-3, TNF-α, IL1 α and IL1 β were 1.0+/−1.0, 16.3+/−10.6, 0.5+/−0.1, 3.9+/−0.1, and 155+/−25.2 pg/mL, respectively. The protein levels of the Ceramic group were 1.0+/−1.0, 10.0+/−0.1, 0.5+/−0.1, 3.9+/−0.1, and 86.3+/−9.6 pg/mL, respectively. The results showed that the IL-1 β protein level was significantly higher for Co-Cr than that for ceramic ($p<0.05$). The MMP-3 protein level also was higher from Co-Cr than from ceramic, however, the difference was not statistically significant. There were no significant differences in MMP-1, IL-1 α, and TNF-α protein levels from culture media between the Co-Cr and Ceramic groups (**Table 2**).

HA of the Co-Cr group was 337+/−38.4 mg/mL. That of the Ceramic group was 257+/−11.1 mg/mL. HA from the Co-Cr group was significantly higher than that from the Ceramic group ($p<0.05$).

One µg of total RNA was converted to cDNA by RT from each sample. Integrated density values of GAPDH in the linear PCR range (40 cycles) of the two groups were the same as integrated density units. There were no significant differences in GAPDH band density between the two groups.

Figure 3 shows the mRNA expression value of the Ceramic group normalized to the value of the Co-Cr group. Expression of type II collagen mRNA was three times higher in the Ceramic group than in the Co-Cr group. IL-1 β mRNA expression in the Ceramic group was one third of that in the Co-Cr group. There were no significant differences in type I collagen, type III collagen, ag-

Table 1 PCR primer ID number and source used in this study

Gene	Entrez gene ID	Transcript accession #	Location on NCBI genome assembly
Collagen I	397571	AF201723.1	26417141
Collagen II	397323	AF201724.1	81533221
Collagen III	100152001	NM_001243297.1; FS680880.1; AK235399.1	NA
Aggrecan	397255	NM_001164652.1; AF314813.1	59457249
TNF-α	397086	NM_214022.1; EU682384.1; X57321.1; JF831365.1; M29079.1	27551378
IL-1α	397094	NM_214029.1; M86730.1; X52731.1	45456913
IL-1β	397122	NM_214055.1	NA
MMP-1	397320	NM_001166229.1; AK231224.1; EU722905.1; X54724.1	37363918
MMP-3	396769	AB044413.1	37399963
MMP-13	397346	AF069643.1	37500581
GAPDH	396823	NM_001206359.1	NA

MMP-3, Matrix metalloproteinase-3; IL-1 α, Interleukin-1 alpha; IL-1 β, Interleukin-1 beta; TNF, Tumor necrosis factor; MMP-1, Matrix metalloproteinase-1

Table 2 The protein level and amount of hyaluronic acid from culture media in each group

	MMP-1 (pg/mL)	MMP-3 (pg/mL)	TNF-α (pg/mL)	IL-1α (pg/mL)	IL-1β (pg/mL)	HA (mg/mL)
Co-Cr	1.0+/–0.1	16.3+/–10.6	0.5+/–0.1	3.9+/–0.1	155+/–25.2	337+/–38.4
Ceramic	1.0+/–0.1	10.0+/–0.1	0.5+/–0.1	3.9+/–0.1	*86.3+/–9.6	*257+/–11.1

Mean+/– SD; Co-Cr, Cobalt Chrome group; Ceramic, Ceramic group; MMP-1, Matrix metalloproteinase-1; MMP-3, Matrix metalloproteinase-3; TNF-α, Tumor necrosis factor alpha; IL-1α, Interleukin-1 alpha; IL-1β, Interleukin-1 beta; HA, hyaluronic acid
*: $p<0.05$

grecan, IL-1 α, MMP-1, MMP-3, MMP-13, nor TNF-α mRNA levels between the two groups.

Discussion

In the present study, we applied compressive / rotational loads to cartilage using two different materials, Co-Cr and alumina-based ceramic, under the same conditions. It was shown that the Co-Cr induced a higher protein level and mRNA expression in IL-1 β, as well as a higher level of HA in the culture medium. Furthermore, mRNA expression of type II collagen was significantly lower in the Co-Cr group.

To our knowledge, this is the first report to compare the biological effects on cartilage between Co-Cr and alumina ceramic heads in direct articulation with cartilage. Furthermore, we consider that the present study is valuable because we showed not only mRNA expression but also protein levels of inflammatory cytokines. We did not measure the protein levels of collagen and aggrecan because it is assumed that extra cellular matrix turnover is very slow to detect the differences during the present experimental period.

IL-1 β plays an important role to activate osteoclasts, lymphocytes, and monocytes. It has been reported that bipolar prosthesis may cause osteolysis, i.e., stem loosening by polyethylene debris, among other factors[17–19]. Furthermore, acetabular erosion has occurred by metal outer heads postoperatively[3,6–8,20]. The release of metal ions and debris can result in biological reactions such as osteolysis and acetabular erosion[21]. In addition to these previous reports, the present study showed that cartilage cells expressed IL-1 β in the early postoperative period. This may indicate that not only wear particles of polyethylene, but also direct stimulation of the Co-Cr head against cartilage will be destructive to the hip joint surface and subchondral bone.

Type II collagen is the main component of joint cartilage. If the chondrocytes do not repair matrix macromolecular abnormalities, the tissue will deteriorate. When the cells cannot restore the matrix, excessive loads become exerted on the chondrocytes, and the tissue will deteriorate. The present study showed that cartilage cells expressed less type II collagen mRNA expression in the Co-Cr group. This result also indicates a detrimental effect of Co-Cr heads to joint cartilage.

HA bonds non-covalently with aggrecan and link pro-

tein. This supramacromolecular complex plays an important role in maintaining cartilage function. HA release from cartilage increases with the progression of osteoarthritis[22–24]. The present study showed that culture medium from the Co-Cr group contained a higher amount of HA, thus the cartilage was more damaged by the Co-Cr head. This is in accordance with the results of Muller et al, who reported that the frictional coefficient of ceramic against fresh cadaveric acetabulum was lower compared to metal[25].

Limitations of the present study are the small sample size and no evaluation of synovial tissue metabolism. The synovial membrane is more susceptible to inflammatory cytokines compared to cartilage tissue. It is assumed that the detrimental effect of Co-Cr to the hip joint will be more accelerated. The toxicity of Co and Cr ions is already known. However, we cannot comment on the influence because the concentration of metal ions was not measured.

In conclusion, the ceramic bipolar head had only a small detrimental effect on cartilage cell metabolism, while larger damage was seen for Co-Cr heads. Thus, the application of a ceramic bipolar might reduce Co-Cr bipolar-related complications.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Asada K, Yoshida K, Shimazu A, Yunoki H, Ishida N: Development of alumina ceramic bipolar hip prosthesis and clinical application. Nihon Seikeigeka Gakkai Zasshi 1987; 61: 155–169.
2. Takaoka K, Nishina T, Ohzono K, Saito M, Matsui M, Sugano N, Saito S, Kadowaki T, Ono K: Bipolar prosthetic replacement for the treatment of avascular necrosis of the femoral head. Clin Orthop Relat Res 1992; 277: 121–127.
3. Coleman SH, Bansal M, Cornell CN, Sculco TP: Failure of bipolar hemiarthroplasty: a retrospective review of 31 consecutive bipolar prostheses converted to total hip arthroplasty. Am J Orthop (Belle Mead NJ) 2001; 30: 313–319.
4. Posada OM, Tate RJ, Grant MH: Toxicity of cobalt-chromium nanoparticles released from a resurfacing hip implant and cobalt ions on primary human lymphocytes in vitro. J Appl Toxicol 2015; 35: 614–622.
5. Shah KM, Wilkinson JM, Gartland A: Cobalt and chromium exposure affects osteoblast function and impairs the mineralization of prosthesis surfaces in vitro. J Orthop Res 2015; 33: 1663–1670.
6. Diwanji SR, Kim SK, Seon JK, Park SJ, Yoon TR: Clinical Results of Conversion Total Hip Arthroplasty After Failed Bipolar Hemiarthroplasty. J Arthroplasty 2008; 23: 1009–1015.
7. Haidukewych GJ, Israel TA, Berry DJ: Long-term survivorship of cemented bipolar hemiarthroplasty for fracture of the femoral neck. Clin Orthop Relat Res 2002; 403: 118–126.
8. Nakata K, Ohzono K, Masuhara K, Matsui M, Hiroshima K, Ochi T: Acetabular osteolysis and migration in bipolar arthroplasty of the hip: five- to 13-year follow-up study. J Bone Joint Surg Br 1997; 79: 258–264.
9. Kim YS, Kim YH, Hwang KT, Choi IY: The cartilage degeneration and joint motion of bipolar hemiarthroplasty. Int Orthop 2012; 36: 2015–2020.
10. Whitehouse MR, Aquilina AL, Patel S, Eastaugh-Waring SJ, Blom AW: Survivorship, patient reported outcome and satisfaction following resurfacing and total hip arthroplasty. J Arthroplasty 2013; 28: 842–848.
11. Hu D, Tie K, Yang X, Tan Y, Alaidaros M, Chen L: Comparison of ceramic-on-ceramic to metal-on-polyethylene bearing surfaces in total hip arthroplasty: a meta-analysis of randomized controlled trials. J Orthop Surg Res 2015; 10: 22.
12. Higuchi Y, Hasegawa Y, Seki T, Komatsu D, Ishiguro N: Significantly lower wear of ceramic-on-ceramic bearings than metal-on-highly cross-linked polyethylene bearings: A 10- to 14-year follow-up study. J Arthroplasty 2016; 31: 1246–1250.
13. Reno C, Marchuk L, Sciore P, Frank CB, Hart DA: Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. Biothechniques 1997; 22: 1082–1086.
14. Majima T, Marchuk LL, Sciore P, Shrive NG, Frank CB, Hart DA: Compressive compared with tensile loading of medial collateral ligament scar in vitro uniquely influences m-RNA levels for aggrecan, collagen type II and collagenase. J Orthop Res 2000; 18: 524–531.
15. Majima T, Lo IKY, Randle JA, Marchuk LL, Shrive NG, Frank CB, Hart DA: Collagen type I mRNA expression in ACL deficient MCL scars versus ACL intact scars. J Orthop Res 2002; 20: 520–525.
16. Natsu-Ume T, Majima T, Reno C, Shrive NG, Frank CB, Hart DA: Menisci of the rabbit knee require mechanical loading to maintain homeostasis: cyclic hydrostatic compression in vitro prevents de-repression of catabolic genes. J Orthop Sci 2005; 10: 396–405.
17. Jacobs JJ, Roebuck KA, Archibeck M, Hallab NJ, Glant TT: Osteolysis: basic science. Clin Orthop Relat Res 2001; 393: 71–77.
18. Katsuyama E, Miyamoto H, Kobayashi T, Sato Y, Hao W, Kanagawa H, Fujie A, Tando T, Watanabe R, Morita M, Miyamoto K, Niki Y, Morioka H, Matsumoto M, Toyama Y, Miyamoto T: Interleukin-1 receptor-associated kinase-4 (IRAK4) promotes inflammatory osteolysis by activating osteoclasts and inhibiting formation of foreign body giant cells. J Biol Chem 2015; 290: 716–726.
19. Kuczkowski J, Sakowicz-Burkiewicz M, Iżycka-Świeszewska E, Mikaszewski B, Pawełczyk T: Expression of tumor necrosis factor-α, interleukin-1α, interleukin-6 and interleukin-10 in chronic otitis media with bone osteolysis. ORL J Otorhinolaryngol Relat Spec 2011; 73: 93–99.
20. Kim KJ, Rubash HE: Large amounts of polyethylene debris in the interface tissue surrounding bipolar endoprostheses: Comparison to total hip prostheses. J Arthroplasty 1997; 12: 32–39.
21. Cooper HJ, Della Valle CJ, Berger RA, Tetreault M, Paprosky WG, Sporer SM, Jacobs JJ: Corrosion at the head-neck taper as a cause for adverse local tissue reactions after total hip arthroplasty. J Bone Joint Surg Am 2012; 94: 1655–1661.
22. Sztrolovics R, Recklies AD, Roughley PJ, Mort JS:

Hyaluronate degradation as an alternative mechanism for proteoglycan release from cartilage during interleukin-1 beta-stimulated catabolism. Biochem J 2002; 362: 473–479.
23. Flannery CR, Hughes CE, Schumacher BL, Tudor D, Aydelotte MB, Kuettner KE, Caterson B: Articular cartilage superficial zone protein (SZP) is homologous to megakaryocyte stimulating factor precursor and Is a multifunctional proteoglycan with potential growth-promoting, cytoprotective, and lubricating properties in cartilage metabolism. Biochem Biophys Res Commun 1999; 27: 535–541.
24. Chockalingam PS, Zeng W, Morris EA, Flannery CR: Release of hyaluronan and hyaladherins (aggrecan G1 domain and link proteins) from articular cartilage exposed to ADAMTS-4 (aggrecanase 1) or ADAMTS-5 (aggrecanase 2). Arthritis Rheum 2004; 50: 2839–2848.
25. Muller LP, Degreif J, Rudig L, Mehler D, Hely H, Rommens PM: Friction of ceramic and metal hip hemiendoprostheses against cadaveric acetabula. Arch Orthop Trauma Surg 2004; 124: 681–687.

(Received, January 26, 2018)
(Accepted, September 3, 2018)