JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 13 (2002) 803–809

Human biological reactions at the interface between bone tissue and polymethylmethacrylate cement

J. X. LU[1,2], Z. W. HUANG[1], P. TROPIANO[2], B. CLOUET D'ORVAL[2], M. REMUSAT[1], J. DEJOU[1], J.-P. PROUST[1], D. POITOUT[2*]
[1]*Laboratoire Interface Matrice Extracellulaire et Biomatériaux (IMEB), Faculté d'Odontologie, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France*
E-mail: imeb@odontologie.univ-mrs.fr
[2]*Service de chirurgie orthopédique et de traumatologie du CHU de l'Hôpital Nord, Chemin des Bourrellys, 13915 Marseille Cedex 20, France*
E-mail: dpoitout@ap-hm.fr

This study investigated cytotoxicity of cement fragments harvested from two prosthesis revisions by the MTT test using L929 fibroblasts and human osteoblasts. The results did not show any toxicity of the extracts prepared after 48 and 78 months implantation. We consider that no MMA monomer has been released from the cement fragments.

Histological studies on undecalcified samples harvested around revising prosthesis from 11 patients were used to evaluate tissue reactions at the bone–cement interface after 2–168 months implantation. Cement and prosthesis particles (5–35 μm) either dispersed or forming a layer were observed. A fibrous tissue layer, osteolysis, and osteonecrosis areas were observed at the interface. Besides, fibroblasts, macrophages, and multinucleated giant cells were also observed. New bone formation with osteoid, osteoblasts, and endochondral ossification with fibrocartilaginous tissue has been observed. The tissue reactions seemed to decrease with time. However, osseous trabeculae fractures were observed in the samples after 19 months. Although we consider that monomer toxicity, exothermic reaction, and particles formation may cause short-term prosthesis loosening; the trabeculae fractures may be due to prosthesis and bone cement micromovements. This fractures and particles formation may cause long-term prosthesis loosening.

Introduction

The use of self-polymerizing bone cement of polymethylmethacrylate (PMMA) to seal prosthetic components to the skeleton was considered as a milestone achievement in development of total joint arthroplasty, due to a better prosthetic stability [1]. From the 1960s to 1980s, this bone cement has been widely used in orthopedics. Fine particles of prepolymerized PMMA are mixed with methylmethacrylate (MMA) monomer. The polymerization process is triggered by the reaction between the benzoyl peroxide in the polymer powder with *N,N*-dimethyl-*p*-toluidine. A radio-opacifier such as barium sulfide or zirconium dioxide, is also added to the powder component. The MMA polymerization is highly exothermic and its temperature can exceed 80 °C [2–4]. PMMA bone cement is actually well tolerated and bone tissue may be formed at its surface [5, 6].

Cell necrosis may occur due to (1) monomer toxicity, (2) high temperature of the cement polymerization, (3) osteonecrosis mediated by inflammatory reaction, (4) osteolysis caused by wear debris formation or (5) impairment of blood circulation in the bone caused by reaming, then, plug of cement [7, 8]. PMMA cement has been shown to affect bone metabolism, which may cause a decrease in its revascularization [9, 10]. The major biological disadvantage of this cement is its poor biocompatibility. Moreover, this cement is neither biodegradable nor colonizable by the bone tissue. All these factors can cause a fibrous tissue formation and an osteonecrosis able to induce micromovements at the bone–cement or cement–prosthesis interfaces. The micromovements, cement disintegration, and prosthesis wear can produce debris particles of bone cement and prosthesis. These particles can cause an osteolysis and stimulate the formation of fibrous tissue leading to a loosening of surgical prosthesis. From a biomechanical point of view, the disparity of the elastic modulus at the

*Author to whom all correspondence should be addressed.

bone–cement–metal interfaces and the expected high stress in the cement mantle are also important factors for the loosening.

The residual MMA monomers is responsible for the irreversible damage to cells and its decrease with time is well known [11–13], but how long is the duration of the monomer releasing after PMMA cement implantation? The aim of this study was to investigate the cytotoxicity of PMMA cement obtained from revising prosthesis, and the tissue reactions at the interface between bone tissue and cement.

Materials and methods

Evaluation of cytotoxicity using MTT test

Two samples of cement fragments from two patients having surgical revision for femoral component prosthesis loosening have been harvested. The first patient was 36 years old, and the revision was performed after 48 months of arthroplasty. The second patient was 65 years old and the revision took place 78 months after arthroplasty. About 20–40 g cement samples from each patient were cleaned with a physiological saline solution. The samples were cut into 0.2–0.5 cm^3 pieces with bone scissors in rigorous aseptic conditions for the next procedures.

The total covered surface was calculated and the extraction vehicle, minimum essential medium (MEM, GIBCOBRL, France) was added to the samples at 3 $cm^2\,mL^{-1}$. After 5 days incubation at 37 °C (first 5 days), the extraction medium was collected and conserved at 4 °C. Then, the samples were recovered by the same quantity of MEM and incubated at 37 °C for another 5 days period (second 5 days). All extraction media were prepared to test the cytotoxicity using murine fibroblasts (L929) and human osteoblasts.

The murine fibroblast cells (ATCC, L929) were cultured in 75 cm^2 polystyrene flasks using MEM supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 2.5 $\mu g\,mL^{-1}$ mycostatin, 100 $UI\,mL^{-1}$ penicillin, 100 $\mu g\,mL^{-1}$ streptomycin ν and were incubated at 37 °C in a 5% CO_2 atmosphere with 95% relative humidity. The 1×10^4 cells were put into each well of 96-well microplates. After 48 h incubation, the medium was discarded and replaced with 200 µl of the extraction medium at different concentrations (1%, 10%, 50%, and 100%).

The human osteoblast-like cells were obtained from cancellous bone tissue of femoral head of a 12-year-old girl. These cells were seeded after a second passage in Dulbecco's Modified Eagle's Medium (DMEM, GIBCOBRL, France) with 20% foetal calf serum, 2 mM L-glutamine, 100 $UI\,mL^{-1}$ penicillin, 100 $\mu g\,mL^{-1}$ streptomycin, 2.5 $\mu g\,mL^{-1}$ mycostatin and were incubated at 37 °C in a 5% CO_2 atmosphere with 95% relative humidity. The concentrations of the cells and extraction medium were similar to the previously described methods.

After 24 h incubation for all microplates, the medium was discarded and the cells washed with 0.2 ml Dulbecco's phosphate buffered solution (PBS). Then PBS was replaced with 0.2 ml per well of 0.5 $mg\,mL^{-1}$ 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT)-sodium succinate solution in PBS and reincubated at 37 °C for 1 h in a 5% CO_2 atmosphere. At the end of the incubation, the excess MTT solution was removed and replaced with 0.2 mL per well of dimethyl sulfoxide, and the optical density was immediately measured in a Metertech ELISA reader at 550 nm. Control cells with fresh medium were used as 100% controls of cell viability. Cytotoxicity was calculated as the percentage of cell viability of control cells.

Evaluation of tissue reactions using histology

Eleven cement samples from 11 patients having surgical revision due to prosthetic loosening used PMMA cement included six total hip prosthesis (THP), four total knee prosthesis (TKP), and one total elbow prosthesis (TEP). The mean patient age at prosthetic revision was about 57 years (range: 21–84 years). The preoperative diagnosis was six cases of osteoarthritis, two cases of fractures, two cases of malign tumor and one case of post-osteomyelitis. The mean interval form the resurfacing arthroplasties to the revision was about 44 months (range: 2–168 months) (Table I).

During the revision surgery, a biopsy of connective-tissue layer and of some bone tissue at the bone–cement interface was performed. The biopsy samples were fixed in 10% buffered formaldehyde (pH 7.2) for 14 days and then rinsed under tap water for 12 h. They were dehydrated through successive alcohol concentrations (80° to absolute) and cleared in toluene, embedded in polymethylmethacrylate. After hardening, the samples were shaped to direct the section perpendicular to the

TABLE I Clinical data on patients

Case (No.)	Gender	Age	Diagnoses	Prosthesis	Duration (months)
1	F	61	Femoral head fracture	THP	2
2	F	74	Polyarthritis	TEP	8
3	F	84	Gonarthrosis	TKP	9
4	F	45	Coxarthrosis	THP	14
5	F	25	Femoral osteosarcoma	TKP	19
6	F	82	Gonarthrosis	TKP	24
7	M	70	Coxarthrosis	THP	26
8	M	21	Knee fracture	TKP	60
9	F	54	Coxarthrosis	THP	72
10	F	56	Pelvis chondrosarcoma	THP	79
11	F	55	Post-osteomyelitis	THP	168

THP, total hip prosthesis; TKP, total knee prosthesis; TEP, total elbow prosthesis.

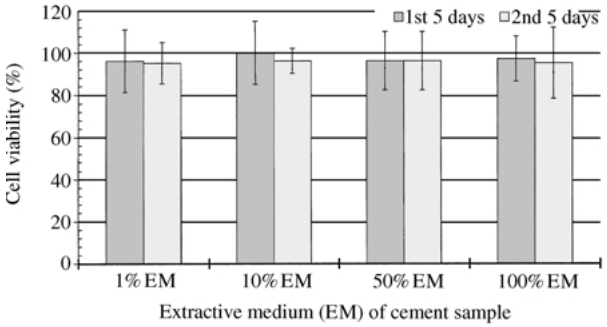

Figure 1 Effect of the medium extracted from the cement of the first patient on fibroblast (L929) viability.

bone–cement interface, and then 200–300 µm-thick sections were cut under cooling water with a sawing microtome (ISOMET 2000, Buehler®, USA). The sections glued onto a plastic support were polished manually (STRUERS, Denmark) to 50 ± 5 µm-thick under cooling water. Finally, they were stained with Van Gieson's Picro-Fuchsine staining. The sections were examined under light microscopy with an eyepiece micrometer (PK 8, Reichert, Austria) to measure particle size and tissue reactions.

Results

Evaluation of cytotoxicity using MTT test

The cytotoxicity of the two cement samples was evaluated by the MTT test using the L929 fibroblasts and human osteoblasts. The extraction medium of the first and second 5 days showed a cell viability more than 87% (Figs. 1–4) which indicated no toxicity of the samples. No significant difference was observed between the tests using the L929 fibroblasts and human osteoblasts, and between the first and second 5 days of extraction medium.

Evaluation of tissue reactions using histology

At the bone-cement interface, four layers of different tissue reactions were observed from the prosthesis to the bone tissue. In the first layer adjacent to the bone cement, debris particles were found with a size ranging from 5 to 35 µm, its were either formed a particle layer from 30 to 150 µm-thick, or dispersed in connective tissue (Fig. 5). In the second layer, connective tissue contained

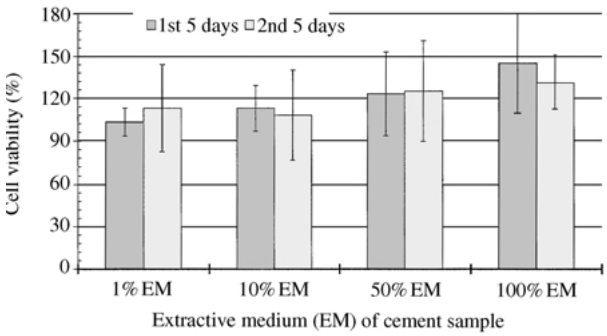

Figure 2 Effect of the medium extracted from the cement of the first patient on osteoblast viability.

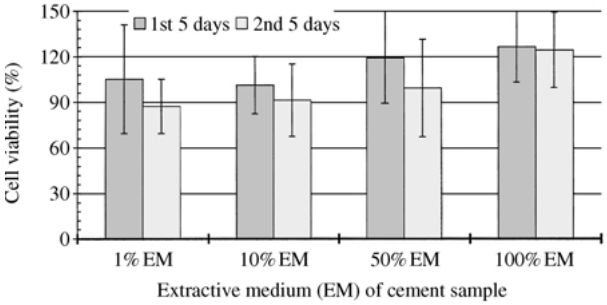

Figure 3 Effect of the medium extracted from the cement of the second patient on fibroblast (L929) viability.

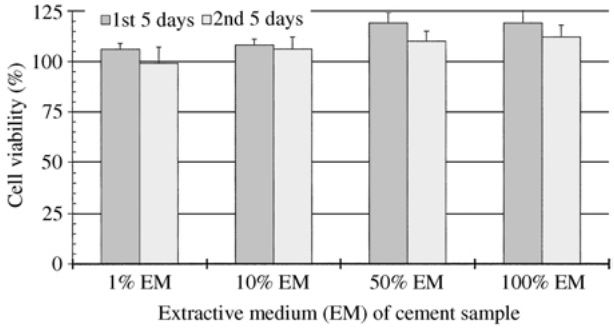

Figure 4 Effect of the medium extracted from the cement of the second patient on osteoblast viability.

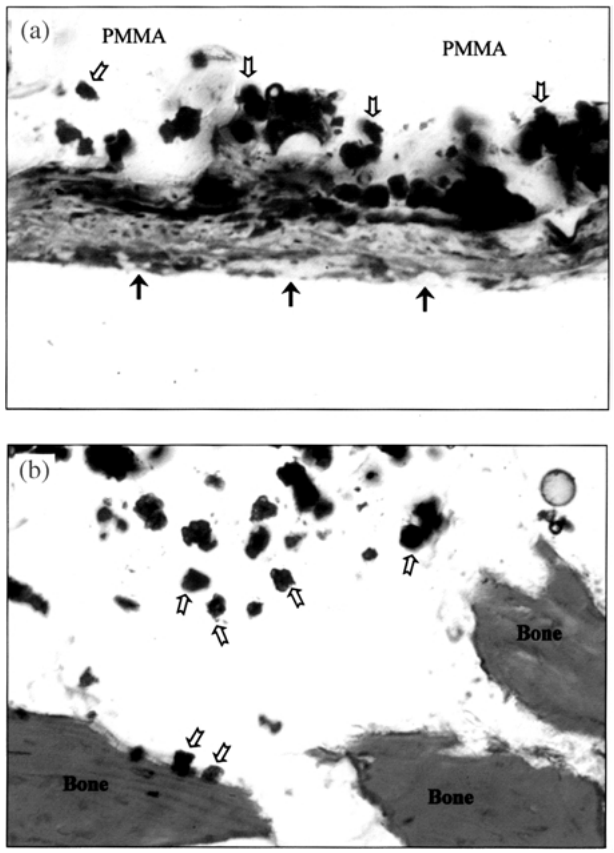

Figure 5 Microscopic examination of the bone–cement interface show debris particles: (a) in case No. 2 (after 8 months of implantation), the debris particles (⇒) formed a layer on the fibrous tissue (→). (b) in case No. 5 (after 19 months), the debris particles (⇒) were found directly on the bone surface since fibrous tissue was not formed (Stain, Van Gieson's Picro-Fuchsine; original magnification × 200).

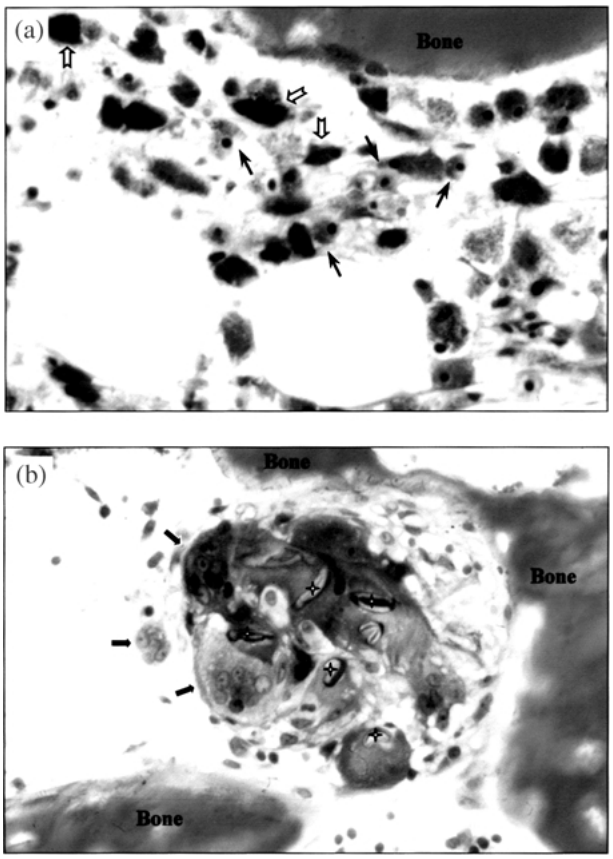

Figure 6 Chronic inflammatory reactions to PMMA bone cement: (a) in case No. 8 (after 60 months of implantation), numerous macrophages (→) and small debris particles (⇒) were found in the bone marrow tissue, (b) in case No. l (after 2 months), large debris particles (◇) were surrounded by multinucleated giant cells (➡) (Stain, Van Gieson's Picro-Fuchsine; original magnification × 400).

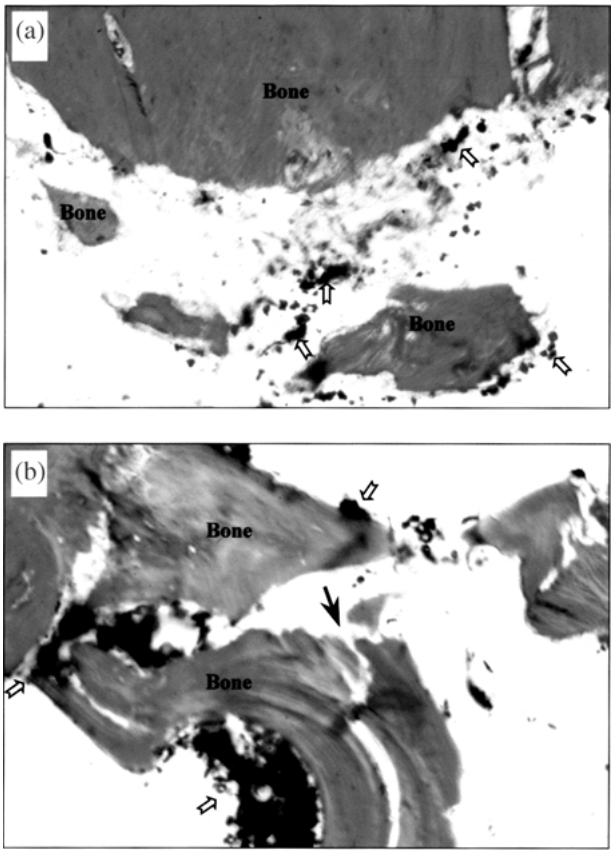

Figure 7 Bone tissue reactions to PMMA bone cement: (a) in case No. l (after 2 months of implantation), the osteolysis area showed numerous small bone fragments without any cells or bone structures but debris particles (⇒) were present at the bone–cement interface, (b) in case No. 8 (after 60 months), trabeculae fracture (→) and penetration of debris particles (⇒) were found in the cancellous bone (Stain, Van Gieson's Picro-Fuchsine; original magnification × 100).

numerous macrophages, or occasional multinucleated giant cells (Fig. 6). Many histiocytes were plumped granular cytoplasm and were arranged in sheets. This layer was 5–850 μm-thick with or without particles and fibroblasts. In the third layer, an osteolysis zone presented numerous small bone fragments without bone structures or cells (Fig. 7(a)). An osteonecrosis zone presented a diminution or an absence of osteocytes and a demineralized bone structure disorganization. There was a fibrous marrow infiltrated with macrophages. Areas of active bone resorption and adjacent area osteogenesis and fibrocartilage formation were also seen in this layer. In the fourth layer, trabeculae fractures were observed in cancellous bone after 19 months implantation (Fig. 7(b)).

The debris particles (ranged from 5 to 35 μm) were found in all 11 cases. The particles formed a layer in six cases, especially in short-term revisions from 2 to 14 months implantation. The connective tissue layer from 5 to 850 μm-thick with macrophages, and the osteonecrosis layer from 5 to 800 μm-thick were observed in all cases. Osteolysis layer was present in six cases, the osteogenesis in two cases and fibrocartilage formation only in one case. The trabeculae fractures were observed in four cases of the eight biopsy samples presenting bone tissue (Table II).

According to the duration of implantation, three groups were distinguished: Group-A $<$ 1 year (three cases), Group-B from 1 to 3 years (four cases), and Group-C $>$ 3 years (four cases). The particle size was always 5–35 μm for each group. The thickness of the particle layer was 30–150 μm and decreased with time. The particle layer presented in all three cases in Group-A, two of four cases in Group-B, and one of four cases in Group-C. The presence of connective tissue layer was constant for all cases in all groups, but its thickness decreased with time: 5–850 μm for Group-A, 50–750 μm for Group-B, and 40–420 μm for Group-C. Macrophages were found in all biopsy samples, but only one case presented multinucleated giant cells.

Because of absence of bone tissue in three biopsy samples (one in Group-A and two in Group-C), bone tissue reactions were observed in only eight samples. The osteonecrosis layer was present in these eight cases, the thickest-one (30–800 μm) was found in Group-A, the thinnest (30–200 μm) in Group-B, and intermediate (40–420 μm) in Group-C. The osteolysis layer was present in one of two cases in Group-A, four of four cases in Group-B and two of two cases in Group-C. Its thickness was Group-B $>$ Group-C $>$ Group-A. The trabeculae fractures shown only after 19 months implantation, and were noted in two of four cases in Group-B and two of two cases in Group-C.

Discussion

MMA monomer release from the PMMA bone cement

The toxicity of PMMA cement has been widely demonstrated. Both MMA, the main component of the

TABLE II The histological results from 11 biopsy samples

Patient	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Duration (month)	2	8	9	14	19	24	26	60	72	79	168
Particles											
Size (μm)	10–35	5–30	10–30	10–30	10–35	5–30	10–35	10–30	5–35	10–30	10–30
Layer thickness (μm)	60–120	30–80	40–90	50–150	−	−	50–100	−	50–100	−	−
Fibrous tissue											
Thickness (μm)	100–850	25–120	5–25	130–200	50–100	150–750	120–470	100–420	50–80	40–100	100–250
Fibroblasts	+ + +	+ + +	+ + +	+	−	−	−	+ +	+ +	−	−
Bone tissue			*							*	*
Necrosis (μm)	200–800	30–50		30–100	30–50	50–200	100–200	300–400	5–10		
Osteolysis (μm)	20–150	−		+	+	+	100–560	50–300	+		
Osteogenesis	+ +	−		−	+	+	−	−	+		
Trabecular fracture	−	−		−	+	−	+	+	+		
Fibrocartilage	+	−		−	−	−	−	−	−		
Inflammation											
Macrophages	+ + +	+ + +	+	+	+	+ +	+	+	+ +	+	−
Giant Cells	+	−	−	−	−	−	−	−	−	−	−

*Case was the absence of bone tissue in biopsy samples.

cement, and its additives can cause irreversible damage to the cells [11]. The MMA toxicity is related to the high quantity of the monomer within the cement. After the polymerization, usually 15 min, about 3–5% of the monomer remains, and decreases to 1–2% with time. These residues are partly eliminated through the blood stream and through breathing, and are partly entrapped *in situ* where they continue to exert a toxic effect. The monomer toxicity should disappear within 4 h and the late effects are to be attributed to the additives in the cement [12]. Granchi *et al*. [13] analyzed the cytotoxicity on the cell cycle of osteoblasts-like cells for five different PMMA cements at 15 and 60 min and 6, 24, and 48 h of the polymerization. The extracts prepared showed a high or moderate toxicity until 6 h polymerization, but no studies were performed after implantation. Our results did not show any toxicity of the extracts prepared with cements after 48 and 78 months implantation. We consider that no MMA monomer has been released from the cement fragments after implantation.

The toxicity disappearance is due to MMA monomer metabolization by the organism. Residual monomers were converted to methylacrylic acid rather than methylester. The methylacrylic acid, as a coenzyme A ester, is a normal intermediate in the catabolism of valine. The existence of an enzyme system would permit methylacrylic acid to enter a normal pathway, leading to carbon dioxide formation. Over 80% of an administered dose of C-labeled MMA is respired as CO_2 within 5–6 h after administration of curing bone cement into living bone [14].

Tissue reactions at the bone–cement interface

In this work, 11 cement samples from 11 patients having revision surgery due to prosthetic loosening were studied by histological technique on undecalcified samples. Their tissue reactions at the bone–cement interface contained: debris particles, connective tissue, osteolysis and/or osteonecrosis, and bone trabeculae fractures. These results correspond to literature results [15–18].

The initial problems of PMMA cement are an irreversible damage to cells and tissues around the cement due to MMA monomer toxicity and high exothermicity during cement polymerization. Tissue necrosis and fibrous tissue formation resulted of these two factors at the bone–cement interface. In this study (Table III), all patients showed these tissue reactions. The thickness of fibrous tissue decreased with time: Group A (5–850 μm) > Group B (50–750 μm) > Group C (4–420 μm). However, the material quality and the surgical techniques can provoke a mechanical instability and formed spaces at the bone–cement interface also leading to fibrous tissue formation. The thickness of the osteonecrosis layer was Group A (30–800 μm) > Group C (5–400 μm) > Group B (30–200 μm), this osteonecrosis was high before 1 year or after 3 years of implantations. We suppose that this osteonecrosis is caused by monomer toxicity, high exothermie of polymerization, surgical trauma, and debris particles formation at less than 1 year of implantation. After 3 years of implantation, the osteonecrosis and the osteolysis are mainly caused by the formation of debris particles.

In this study, the debris particles 5–35 μm in size were observed at the bone–cement interface in all cases. However, 6 of 11 cases formed a particles layer from 30 to 150 μm-thick, especially, all three cases of Group A. The formation of debris particles is caused by PMMA cement disintegration and micromovements, as well as wear of prosthetic polyethylene cup, in the induction of bone resorption [17]. The initial event can be either cement disintegration or wear from articulating surface. Phagocytosis and the development of granulomas lead to osteolysis of the anchoring bone, thus disintegration or wear is enhanced and accelerate the progress of osteolysis. Large particles (up to several hundred microns) will be phagocytosed or surrounded by giant cells. The small particles (lower than a hundred micron)

TABLE III The tissue reactions to PMMA cement in function of the time

	Group-A	Group-B	Group-C
Implanted duration	< 1 year	$1 \sim 3$ years	> 3 years
Case	3	4	4
Formed particles	3 of 3 cases	4 of 4 cases	4 of 4 cases
	ϕ 5–35 μm	ϕ 5–35 μm	ϕ 5–35 μm
Particles layer	3 of 3 cases	2 of 4 cases	1 of 4 cases
	thick: 30–120 μm	thick: 50–150 μm	thick: 50–100 μm
Fibrous tissue	3 of 3 cases	4 of 4 cases	4 of 4 cases
	thick: 5–850 μm	thick: 50–750 μm	thick: 40–120 μm
Fibroblasts	3 of 3 cases	1 of 4 cases	2 of 4 cases
Osteonecrosis	2 of 2 cases*	4 of 4 cases	2 of 2 cases*
	thick: 30–800 μm	thick: 30–200 μm	thick: 5–400 μm
Osteolysis	1 of 2 cases*	4 of 4 cases	2 of 2 cases*
	thick: 20–150 μm	thick: 100–560 μm	thick: 50–300 μm
Osteogenesis	1 of 2 cases*	2 of 4 cases	1 of 2 cases*
Trabecular fracture	0 of 2 cases*	2 of 4 cases	2 of 2 cases*
fibrocartilage	1 of 2 cases*	0 of 4 cases	0 of 2 cases*
Macrophages	3 of 3 cases	4 of 4 cases	3 of 4 cases
Giant cells	1 of 3 cases	0 of 4 cases	0 of 4 cases

*Case was the absence of bone tissue in biopsy samples.

will be ingested by macrophages and will provoke a physiological response termed ''activation'', in which the cell becomes enlarged and releases numerous inflammatory and potentially osteolytic factors [19]. Kobayashi's work [20] showed the debris particle size of 18 patients ranged from 0.48 to 1.32 μm and 75% of the particles were less than 1 μm. Our study observed that the size of the extracellular particles ranged from 5 to 35 μm at the cement–bone interface including the cement surface, and their adjacent soft and bone tissues using light microscopy. In one hand, Kobayashi studied the intracellular and extracellular particles in the adjacent soft tissue. However, they considered there was no correlation between the presence or absence of osteolysis and the morphology of the present particles. There was a highly significant association between the number of particles and the presence of osteolysis.

Particle debris and cell death are well-known as activators of macrophages and can trigger further macrophage cell recruitment, and the release of osteolytic factors, one of which is interleukin 1 (IL-1) [21, 22]. While IL-1 may have bone resorptive activity, bone resorption is believed to be largely mediated by tumor necrosis factor alpha (TNF-α), which is also known as osteoclast activating factor (OAF), a cytokine largely secreted by macrophages. Macrophages can induce bone loss by the release of mediating factors such as OAF, as well as directly, by the release of oxide radicals and hydrogen peroxide [7].

In this study, the trabeculae fractures in cancellous bone adjacent to the cement were found after 19 months of implantation (0 case in Group A, two of four cases in Group B, and two of two cases in Group C). These fractures are due to bone fragility caused by bone resorption, and micromovements of cement and prosthesis. Therefore, we suppose that monomer toxicity, exothermic reaction, and particles formation cause the short-term prosthesis loosening. The osseous trabeculae fractures because of the micromovements of prosthesis and bone cement cause the long-term prosthesis loosening.

References

1. J. CHARNIEY and J. KETTELWELL, *J. Bone Joint Surg. [Br.]* **47** (1965) 56.
2. R. MONGIORGI, G. VALDRE, R. GIARDINO, G. MAGGI, C. PRATI and G. BERTOCCHI, *Bull. Soc. Ital. Biol. Sper.* **69**(1993)365.
3. A. T. BERMAN, J. S. REID, D. R JR. YANICKO, G. C. SIH and M. R. ZIMMERMAN, *Clin. Orthop.* **186** (1984)284.
4. J. B. PARK, R. C. TURNER and P. E. ATKINS, *Biomater. Med. Devices Artif. Organs.* **8** (1980) 23.
5. L. A. THOMSON, F. C. LAW, K. H. JAMES, C. A MATTHEW and N. RUSHTON, *Biomaterials.* **13** (1992)811.
6. A. PIZZOFERRATO, G. CIAPETTI, S. STEA and A. TONI, *Clin. Mater.* **7** (1991) 51.
7. H. C. AMSTUTZ, P. CAMPBELL, N. KOSSOVSKY and I. C. CLARKE, *Clin Orthop.* **276** (1992) 7.
8. W. G. SORENSEN, J. D. BLOOM and P. J. KELLY, *J. Bone Joint Surg. [Am.]* **61** (1979) 417.
9. S. B. CHRISTENSEN, *Acta Orthop. Scand. Suppl.* **214** (1985) 1.
10. J. STURUP, J. MADSEN, E. TONDEVOLD and J. S. JENSEN, *Acta Orthop. Scand.* **61** (1990) 143.
11. J. DE WAAL MALEFIJT, T. J. SLOOF and R. HUISKES, *Acta Orthop. Belg.* **53** (1987) 52.
12. C. M. SCHOENFELD, G. J. CONARD and E. P. LAUTENSCHLAGER, *J Biomed. Mater. Res.* **13** (1979) 135.
13. D. GRANCHI, S. STEA, G. CIAPETTI, L. SAVARINO, D. CAVEDAGNA and A. PIZZOFERRATO, *Biomaterials* **16** (1995) 1187.
14. D. H. CROUT, J. A. CORKILL, M. L. JAMES and R. S. LING, *Clin. Orthop.* **141** (1979) 90.
15. L. N. JENSEN, J. STURUP, M. KRAMHOFT and J. S. JENSEN, *Acta Orthop. Belg.* **57** (1991) 254.
16. J. CHARNIEY, *J Bone Joint Surg. [Br.]* **52** (1970) 340.
17. H. G. WILLERT, H. BERTRAM and G. H. BUCHHORN, *Clin. Orthop.* **258** (1990) 108.
18. M. JASTY, W. JIRANEK and W. H. HARRIS, *Clin. Orthop.* **285** (1992) 116.

19. N. KOSSOVSKY, K. LIAO, D. MILLETT, D. FENG, P. A. CAMPBELL, H. C. AMSTUTZ, G. A. FINERMAN, B. J. THOMAS, D. J. KILGUS, A. CRACCHIOLO and V. ALLAMEH, *Clin. Orthop.* **263** (1991) 263.
20. A. KOBAYASHI, M. A. FREEMAN, W. BONFIELD, Y. KADOYA, T. YAMAC, N. AL-SAFFAR, G. SCOTT, P. A. REVELL, *J. Bone Joint Surg. [Br.]*, **79** (1997) 844.
21. S. R. GOLDRING, A. L. SCHILLER, M. ROELKE, C. M. ROURKE, D. A. O'NEIL and W. H. HARRIS, *J. Bone Joint Surg. [Am.]*, **65** (1983) 575.
22. P. STASHENKO, F. E. DEWHIRST, W. J. PEROS, R. L. KENT and J. M. AGO, *J. Immunol.* **138** (1987) 1464.

Received 19 June 2000
and accepted 4 February 2002