No effect of methacrylate-based bone cement CMW 1 on the plasmatic phase of coagulation, red blood cells and endothelial cells in vitro

Elisabetta Cenni[1], Gabriela Ciapetti[1], Donatella Granchi[1], Susanna Stea[1], Lucia Savarino[1], Alessandra Corradini[1] and Alessandro Di Leo[2]

[1]Dipartimento Putti, Istituti Ortopedici Rizzoli, via di Barbiano 1/10, IT-40136 Bologna, Italy. Tel +39 0516366748. E-mail: elisabetta.cenni@ior.it; [2]Istituto di Fisiopatologia della Riproduzione, Policlinico S. Orsola, Bologna, Italy
Submitted 00-01-23. Accepted 00-10-08

ABSTRACT – The compatibility of a methacrylate-based bone cement (CMW 1, DePuy International Ltd, England) used for the fixation of joint prostheses was evaluated on plasma, an erythrocyte suspension and cultured human endothelial cells. The extract of the cement was tested, following 1 hour and 7 days of curing. After the contact in vitro of the extract with plasma, activated partial thromboplastin time, antithrombin III, thrombin-antithrombin complexes and fibrin degradation products were assayed. Hemolytic activity was tested by adding the cement extracts to a suspension of erythrocytes. After 4 hours of incubation at 37 °C, the hemoglobin concentration was determined on the supernatants by the colorimetric method. The effect of the cement on tissue factor and thrombomodulin production was evaluated on human umbilical vein endothelial cell cultures. Tissue factor was determined in cell lysates by enzyme immunoassay, following 4 hours' incubation of cultures with the cement extract. Thrombomodulin was assayed in cell lysates by enzyme immuno assay, after 24 hours' incubation with the cement extract. The response to all trans-retinoic acid (ATRA) was tested.

The cement caused no significant modifications of the coagulation tests, had no hemolytic activity, did not determine tissue factor production and did not modify thrombomodulin, compared to the negative control. The response to stimulation with ATRA was similar to that of the negative control. We conclude that the cement extract does not affect the plasmatic phase of coagulation, has no effect on erythrocytes, does not induce the expression of procoagulant activity by endothelial cells and does not impair their antithrombotic property, within the limits of the tests performed.

■

It has been shown that polymethylmethacrylate (PMMA), a basic component of bone cements, is very toxic, because of the presence of residual monomer and of stabilizers, catalyzators and other components which may be released. The bone cement caused vascular and bone reactions in rabbits in vivo (Albrektsson and Linder 1984).

A careful biocompatibility evaluation is necessary, owing to the high incidence of thromboembolic disease after hip replacement (Haake and Berkman 1989). Although surgical trauma and hospitalization may contribute to the onset of thrombosis, a role of the prosthesis biomaterials cannot be excluded a priori. On the basis of clinical studies, the highest risk for thrombosis seems to be connected with the insertion of the cemented femoral component (Sharrock et al. 1995).

Postoperative deep-vein thrombosis (DVT) may be related also to the local and systemic effects of monomer of methylmethacrylate (MMA) released into the circulation from curing cements by activation of coagulation (Dahl et al. 1992) and damage to blood cells and endothelial cells (Dahl et al. 1994). The toxicity of cements is linked not only to the liquid monomer component of MMA, but also to other chemicals that can be released during polymerization.

To demonstrate the effect of the substances released by cement on coagulation, we determined the effect on the plasmatic phase of hemostasis, and the damage to erythrocytes and endothelial cells in vitro.

Material and methods

Material

A methacrylate-based bone cement used in orthopedics for the fixation of joint prostheses (CMW 1, DePuy International Ltd, Blackpool, England) was treated according to the manufacturer's instructions. The composition of the cement (% weight/weight) was as follows: powder, 88.85% polymethylmethacrylate; 2.05% benzoyl peroxide; 9.10% barium sulfate; liquid, 98.215% methylmethacrylate; 0.816% N,N dimethylparatoluidine; 0.945% ethylic alcohol; 0.022% ascorbic acid; 0.002% hydroquinone.

The powder was mixed with the solution for the period suggested by the manufacturer, then the cement was allowed to cure for 1 hour and 7 days. Extraction was performed according to EN ISO 10993-12 standard (1996), i.e., 0.2 g of material/mL of extraction medium and incubation at 37 °C for 72 hours. The extraction liquid was saline for the coagulative tests, phosphate-buffered saline (PBS) for the hemolysis test and culture medium for the experiments on cell cultures. Controls were obtained by extracting respectively saline, PBS and medium, on the same conditions as the cement.

Coagulative tests

0.9 mL of platelet-rich plasma (PRP), obtained by centrifugation from citrated venous blood from healthy donors, were put in siliconized tubes containing 0.1 mL of the cement extracts. The negative control was PRP placed in contact with saline extract. The tubes were placed in a mixer for 30 minutes at room temperature. After the contact-period, PRP was centrifuged at 1500 × g for 15 minutes, then activated partial thromboplastin time (APTT), antithrombin III, thrombin-antithrombin complexes (TAT) and fibrin degradation products (FbDP) were determined.

APTT was determined using a reagent of micronized silica and phospholipids from rabbit brain (Automated APTT, Organon Teknika, Morris Plains, New Jersey, USA) and a 0.025 M calcium chloride solution (Biomérieux, Marcy l'Étoile, France).

Antithrombin III activity was determined by a chromogenic method (MDA Antithrombin III, Organon Teknika). The results were expressed as activity percentage, assuming as 100 % the activity of a normal calibration plasma (Calibration Plasma, Instrumentation Laboratory, Lexington, MA, USA).

The concentration of TAT was determined by enzyme immunoassay with polyclonal antibodies (Behringwerke AG, Marburg, Germany).

Fibrin degradation products (FbDP) were determined by enzyme immunoassay (Fibrinostika FbDP, Organon Teknika) with a monoclonal antibody specific for fibrin degradation products.

Hemolysis test

Erythrocytes from human venous blood anticoagulated with a mixture of citrate, phosphate and dextrose (CPD) were washed and diluted 1:9 with PBS. 5.0 mL of the suspension were incubated with 4.0 ml of the extracts or PBS alone (negative control) at 37 °C for 4 hours without agitation. After incubation, the suspension was centrifuged and the hemoglobin concentration was determined on the supernatants, with a colorimetric method based on the catalytic action of hemoglobin in the oxidation of 3, 3', 5, 5'-tetramethylbenzidine by hydrogen peroxide (Hemoglobin Plasma, Sigma, St. Louis, Missouri, USA).

Endothelial cells

Endothelial cells were prepared from the umbilical cord vein. The vein was flushed with saline to remove blood and then filled with a collagenase solution (Type IV-S, Sigma) for 15 min at 37 °C. Detached cells were recovered by flushing with a buffer consisting in 0.14 M sodium chloride (Carlo Erba, Rodano, Milan, Italy), 0.004 M potassium chloride (Riedel-de-Haen, Seelze, Germany), 0.001 M glucose (Carlo Erba), 0.001 M potassium monobasic phosphate (Carlo Erba, Rodano, Milan, Italy), 0.01 M sodium bibasic phosphate (Carlo Erba). The cells were resuspended in culture medium, an equal mixture of medium RPMI 1640 (Mascia Brunelli, Milan, Italy) and medium 199 (Sigma), supplemented with 25 mM Hepes buffer (Sigma), 2 mM L-glutamine (Biological Industries, Kibbutz Beit-Haemek, Israel), 100 IU/mL of penicillin (Irvine Scientific, Santa Ana, California, U.S.A.), 100 μg/mL of streptomycin (Irvine Scientific), and 20% Fetal Calf Serum (FCS)

(Boehringer Mannheim GmbH, Mannheim, Germany). The cells were seeded in a tissue culture-treated polystyrene flask and incubated at 37 °C in humidified air with 5% CO_2. The medium was refreshed 3 times a week. The experiments were performed on cultures at the second passage.

Neutral red test

To establish the extract dilution which had the least cytotoxic effect, the neutral red test (Hansen et al. 1989) was performed on endothelial cells after 4 and 24 hours of incubation with the cement extracts undiluted and diluted 1:2, 1:4, 1:8, 1:16 and 1:32 with medium. Other cultures were incubated for 24 hours with absolute ethanol diluted 1:16000; 1:1600; 1:160; 1:16 with medium. At the end of the incubation period and after the cells had been washed, the remaining cells were incubated with 0.4% neutral red dye (Color Index 50040) dissolved in distilled water and then diluted in medium to yield a final concentration of 50 μg/mL. After 2 hours of incubation at 37 °C, the cells were disrupted, the color intensity was read at 540 nm and the viability of samples was calculated as a percentage of the negative control (i.e., cells incubated with medium alone). The extract dilution was considered toxic if the viability percentage was ≤ 80.

Tissue factor assay

Endothelial cells were seeded on 1.0 cm diameter tissue culture-treated polystyrene wells, at a concentration of $0.5–2.5 \times 10^5$/mL. The cultures were incubated at 37 °C for 72 hours, but the medium was refreshed after 24 hours. 2 mM L-glutamine, 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 20% FCS were added to the cement extract. 2 mL of the cement extract were added to each well. The negative control was the extracted medium, to which glutamine, antibiotics and FCS were added after thawing. A solution of endotoxin (from Escherichia coli 055:B5, Sigma, St. Louis, Missouri, USA) in extracted medium at a final concentration of 10 μg/mL was the positive control. The cultures were incubated for 4 hours at 37 °C, then the cells were disrupted by three cycles of freeze-thawing.

Tissue factor was determined by enzyme immunoassay with a monoclonal antibody against human tissue factor (Imubind Tissue Factor ELISA Kit, American Diagnostica Inc., Greenwich, CT, USA). The optical density was read at 450 nm. The optical density of the "blank" was subtracted from standards and samples, then the calibration curve was calculated, according to the function of polynomial regression. The samples with levels of tissue factor higher than the most concentrated standard were diluted 1:5 in PBS; the values obtained were multiplied by the dilution factor. The concentrations, expressed as pg/mL, were divided by the number of cells in the inoculum, to obtain the tissue factor as pg/cell.

Thrombomodulin assay

The cells were seeded on 1.0 cm diameter tissue culture-treated polystyrene wells, at concentrations of $0.6–1.5 \times 10^5$/mL. The cultures were incubated at 37 °C for 48 hours, and the medium was refreshed daily. 2 mM L-glutamine, 100 IU/mL of penicillin, 100 μg/mL of streptomycin and 20% FCS were added to the cement extract. 0.4 mL of cement extract were added to each well. The negative control was the extracted medium, to which glutamine, antibiotics and FCS were added after thawing. A solution of all trans-retinoic acid (ATRA) (Sigma, St. Louis, Missouri, USA) at a final concentration of 10 μmol/L, was added to one aliquot of each cement extract and extracted medium. ATRA had been previously dissolved in absolute ethanol to a concentration of 832 mmol/L. With the exception of the wells with ATRA, 1.0 μL of absolute ethanol was added to all subcultures. The cells were incubated at 37 °C for 24 hours.

After the incubation period, the cells were lysed with 0.5% Triton X100 (Sigma) in PBS. Thrombomodulin in cell lysates was measured by enzyme immunoassay, using monoclonal antibodies (Asserachrom Thrombomodulin, Diagnostica Stago, Asnières-sur Seine, France). The samples were diluted 1:5 with the dilution buffer in the kit. The optical density was read at 492 nm. The calibration curve was calculated, according to the function of linear regression after logarithmic transformation. The values were multiplied to obtain the volume of the cell lysate and divided by the number of cells in the inoculum, so as to convert thrombomodulin into pg/cell.

Table 1. Hematologic tests after contact with bone cement extract following 1 hours' and 7 days' curing. Values are the mean (SEM) of 10 experiments (5 experiments for the assay of plasmatic hemoglobin). The negative controls were plasma in contact with saline extract for APTT, antithrombin III, TAT complexes and FbDP and an erythrocyte suspension incubated with PBS extract for plasmatic hemoglobin assay

Test	Unit of measure	Negative control	Bone cement after 1 hrs' curing	P-value [a]	Bone cement after 7 days' curing	P-value [a]
APTT	seconds	37 (0.8)	37 (0.6)	0.5	37 (0.7)	0.6
Antithrombin III	%	113 (3.6)	116 (4.8)	0.6	104 (3.2)	0.1
TAT complexes	μg/L	7.3 (0.8)	8.2 (0.8)	0.4	7.5 (0.8)	0.9
FbDP	ng/mL	130 (13)	120 (14)	0.7	146 (23)	0.6
Plasmatic hemoglobin	mg/dL	2.4 (0.8)	3.5 (1.0)	0.4	3.4 (1.0)	0.4

[a] vs. control

Statistics

The results were expressed as the arithmetic mean (SEM). The statistical analysis of the effect of the cement extracts on clotting tests and the hemolysis test was done with analysis of variance (ANOVA), and Bonferroni-Dunn's multiple comparison test was used to detect specific differences between groups. $P < 0.002$ was considered statistically significant in the clotting tests; $p < 0.0002$ was considered statistically significant in the hemolysis test. The effects on tissue factor and thrombomodulin expression were estimated with the Mann-Whitney U-test. A p-value of < 0.05 was considered statistically significant in the Mann-Whitney U-test.

Results

Coagulative tests

The extracts of bone cement cured for 1 hour or 7 days caused no significant variations in the tests. APTT, after contact with the cement extract, was similar to that in the negative control. Antithrombin III showed an insignificant decrease following contact of plasma with the cement extract after 7 days of curing. TAT levels increased, but not significantly after contact with the extract after 1 hour of curing. FbDP showed no remarked change after contact with the cement extracts (Table 1).

Hemolysis test

The extracts had no hemolytic effect on the human red cell suspension: the hemoglobin concentration in the supernatants was similar to that in the control (PBS) (Table 1).

Cytotoxicity test

We found only a slight reduction in the percentage viability in cell cultures after contact with bone cement, both after 1 hour and 7 days of curing, regardless of the dilution. No cytotoxic effect was detected even when we used absolute ethanol (Table 2).

Tissue factor and thrombomodulin assay

Tissue factor did not increase significantly in the cell lysates challenged with cement extract, cured for 1 hour or 7 days, compared to the negative control. The concentration of tissue factor increased in cultures stimulated by endotoxin.

Thrombomodulin levels showed no significant change after contact with cement extracts, but a highly significant increase was observed after stimulation with ATRA. ATRA caused significant increases when added to the control cultures and to the cultures incubated with cement extracts (Table 3).

Discussion

Patients undergoing major joint replacement are at high risk of postoperative DVT and pulmonary embolism, even if antithrombotic prophylaxis is given (Dahl 1998, 1999). The high incidence of thromboemboli after a hip prosthesis may be linked not only to the surgical trauma, but also to the graft material.

Table 2. Cytotoxicity (expressed as percent viability) of various concentrations of cement extracts after 1 hours' and 7 days' curing, and of absolute ethanol. Values are the mean (SEM) of 3 replicates

Material	Incubation time (hour)	Dilution					
		Undiluted	1:2	1:4	1:8	1:16	1:32
Bone cement after 1 hrs' curing	4	98 (6.0)	115 (4.4)	110 (1.7)	114 (4.2)	109 (4.6)	114 (7.6)
	24	121 (6.2)	151 (12)	138 (1.4)	151 (5.7)	159 (6.4)	148 (10)
Bone cement after 7 days' curing	4	100 (4.2)	100 (8.4)	101 (7.0)	99 (8.7)	99 (4.9)	92 (4.4)
	24	181 (0.6)	192 (0.3)	188 (3.7)	188 (6.3)	189 (3.6)	224 (20)
Material		Dilution					
			1:16	1:160	1:1600	1:16000	
Absolute ethanol	24		119 (16)	156 (4.7)	156 (1.7)	150 (5.8)	

Table 3. Tissue factor and thrombomodulin after contact with bone cement extract following 1 hours' and 7 days' curing. Values for tissue factor are the mean (SEM) of 10 experiments; values for thrombomodulin are the mean (SEM) of 9 experiments

Material	Tissue factor (pg/cell) × 10^{-3}	P-value [a]	Thrombomodulin pg/cell	P-value
Negative control	0.17 (0.03)	–	0.18 (0.01)	–
LPS	10.7 (2.67)	0.0002	–	–
ATRA	–	–	0.42 (0.05)	0.0003 [a]
Bone cement after 1 hrs' curing	0.09 (0.02)	0.06	0.19 (0.02)	0.7 [a]
Bone cement after 1 hrs' curing + ATRA	–	–	0.58 (0.10)	0.002 [b]
Bone cement after 7 days' curing	0.16 (0.04)	0.4	0.21 (0.03)	0.5 [a]
Bone cement after 7 days' curing + ATRA	–	–	0.57 (0.11)	0.0009 [c]

[a] vs. control.
[b] vs. bone cement after 1 hrs' curing.
[c] vs. bone cement after 7 days curing.

Prospective studies suggest that DVT may occur more often in cemented joint arthroplasties (Hogevold et al. 1990). During surgery, marked activation of coagulation was seen after inserting the cemented femoral component (Sharrock et al. 1995). The risk of developing DVT long after surgery was ascribed to release of small amounts of MMA locally for several days, in conjunction with the acute effect of large doses of MMA released during the initial curing phase (Dahl et al. 1997).

However, reports concerning the high incidence of DVT in cemented arthroplasties are conflicting. Laupacis et al. (1996) suggested that the higher incidence of DVT with cemented prostheses was associated with the higher mean age of the patients. Clarke et al. (1998) showed a greater prevalence of DVT after uncemented prostheses, but significantly larger thrombi after replacements with cemented knees.

The high incidence of thromboemboli in hip prostheses may be related to surgical trauma, more than to the graft material. However, cement may damage endothelial cells by releasing chemicals, such as MMA, benzoyl peroxide, or N,N-dimethyl-paratoluidine. Barium sulfate also may result in cell death after long exposure and large doses (Rae 1977).

The notion that cement may contribute to post-surgical DVT justifies the study of its blood compatibility. Some of the MMA, which still remains free after polymerization, is partly trapped nearly, where it can induce local cytotoxic effects (Schoenfeld et al. 1979), and the rest enters venous blood (Dahl et al. 1992).

We tested blood compatibility of bone cement CMW 1, using assays to explore the coagulation system and endothelial cell viability and its functions. Although in vitro research cannot replace clinical studies, the former permit the effect of the artificial material to be distinguished from that due to surgery.

Cement extracts were tested because more easily standardized assays could be used, especially for materials difficult to handle in small amounts, such as cement. Moreover in our opinion, the toxic effect of the chemicals released is more important than the physical characteristics of the surface. CMW 1 was polymerized in our laboratory in accord with the recommendations of the manufacturer, to obtain a mixture as nearly as possible similar to the cement prepared by the surgeon. A single preparation of each extract was made under uniform conditions of polymerization, curing and extraction. CMW 1 was extracted using aqueous solvents (saline, PBS, culture medium) to simulate monomer release into the interstitial fluids surrounding the reacting cement mass in the body. The pH of the extracts was 7.0–7.5. If non-aqueous solvents were used, the experimental conditions would differ even more than the in vivo situation. The variations induced by the CMW 1 extract were compared to the results obtained by putting the plasma or the cells in contact with the extraction medium alone, which was exposed to the same extraction conditions as the bone cement. The levels of the cement components in the extract solutions were not determined. This extraction method has been standardized for some time. With this method, the silicone effect was evaluated in L929 cells (Ciapetti et al. 1998), the toxicity of some dental cements for MG63 cells was shown (Granchi et al. 1995) and bone cement-induced apoptosis or necrosis was determined in the HL60 line (Ciapetti et al. 2000a).

Cement extracts were prepared after 1 hour's and 7 days' curing. The 1-hour curing was chosen to evaluate the cement effects immediately after implant. The shorter time involved less standardization of the manual operations concerning extract preparation. The 7-day curing time was chosen to evaluate the effect of wholly polymerized cements.

The effect of CMW 1 on the plasmatic phase of coagulation was evaluated by APTT and other tests assessing thrombin generation. TAT generation has also been used to determine blood compatibility of other orthopedic materials, such as titanium (Hong et al. 1999).

The cytotoxicity of cement extracts was tested in preliminary experiments with the quantitative version of the neutral red uptake test. We wished to study the effect of cement on the functions of viable cells; the function impairment observed for toxic concentrations could be due to cell death. Dahl et al. (1994) studied the effect of cement on endothelial cells also by scanning electron microscopy. Within minutes after adding MMA, they found that the endothelial cells lost their polygonal shape, became round, and were no longer adherent to one another and to the substratum. Probably this difference is linked to the fact that they tested the monomer of methylmethacrylate, while we tested the polymerized cement, cured for 1 hour. Indeed, our aim was to study the effect of the polymerized cement after the implantation.

Not all cements have the same cytotoxic effect. In a previous study, the bone cements Cerim LT and CMW 2 were cytotoxic for L929 cells. Cemex Isoplastic, Zimmer-Low viscosity and CMW 1 caused only a slight reduction in cell viability (Ciapetti et al. 2000b). CMW 1 extract did not affect endothelial cell and erythrocyte viability. Probably, the cytotoxic effect differs in various cell types, depending on the stage of differentiation. Endothelial cells are more specialized and proliferate less actively than the established line L929.

We studied the effect of CMW 1 on endothelial cell function by evaluating tissue factor production and thrombomodulin release. Damaged endothelial cells express tissue factor activity and reduce thrombomodulin. CMW 1 did not affect the tissue factor or thrombomodulin release from endothelial cells and did not impair the response to ATRA.

In other experiments, we evaluated the effect of CMW 1 on peripheral blood mononuclear cells. CMW 1 did not affect viability significantly, but increased the percentage of pre-apoptotic cells (Granchi et al. 2000a). Moreover, CMW 1 did not affect the release of TNFα, IL-6 and GM-CSF (Granchi et al. 2000b).

According to the literature, MMA may cause procoagulant phospholipids to move outside the cell membrane (Dahl 1997a), and it affects endothelial lesions, tissue factor production by monocytes and platelet activation (Dahl 1997b). Our study on polymerized CMW 1 showed neither activation of the plasmatic phase nor endothelial damage.

We might suppose that the differences in cement toxicity, which are probably related to the chemical composition, may explain the discrepancies between the reports concerning the incidence of DVT in cemented arthroplasties. During polymerization, all cements release methacrylate monomer, which is cytotoxic for endothelium and activates monocytes and platelets (Dahl 1997b). After polymerization, some cements may still be toxic for endothelium, while others are not. After 7 days of curing, only a few cements are still toxic, as shown by Ciapetti et al. (2000b).

We conclude that the CMW 1 extract does not affect the plasmatic phase of coagulation or erythrocytes, does not induce the expression of procoagulant activity by endothelial cells and does not impair their antithrombotic effects within the limits of the tests performed. Therefore, it can be said that the onset of deep venous thromboses after joint replacements is linked to factors other than the presence of this type of cement.

This research was partly supported by grants from Istituti Ortopedici Rizzoli, 'Ricerca Corrente'.

Albrektsson T, Linder L. Bone injury caused by curing bone cement. A vital microscopic study in the rabbit tibia. Clin Orthop 1984; 183: 280-7.

Ciapetti G, Granchi D, Stea S, Savarino L, Verri E, Gori A, Savioli F, Montanaro L. Cytotoxicity testing of materials with limited in vivo exposure is affected by the duration of cell-material contact. J Biomed Mater Res 1998; 42: 485-90.

Ciapetti G, Granchi D, Cenni E, Savarino L, Cavedagna D, Pizzoferrato A. Cytotoxic effect of bone cements in HL-60 cells: distinction between apoptosis and necrosis. J Biomed Mater Res 2000a; 52: 338-45.

Ciapetti G, Granchi D, Stea S, Cervellati M, Pizzoferrato A. In vitro testing of ten bone cements after different time intervals from polymerization. J Biomater Sci Polym Ed 2000b; 11: 481-93.

Clarke M T, Green J S, Harper W M, Gregg P J. Cement as a risk factor for deep-vein thrombosis. Comparison of cemented TKR, uncemented TKR and cemented THR. J Bone Joint Surg (Br) 1998; 80: 611-3.

Dahl O E. Cardiorespiratory and vascular dysfunction related to major reconstructive orthopedic surgery. Acta Orthop Scand 1997a; 68: 607-14.

Dahl O E. The role of the pulmonary circulation in the regulation of coagulation and fibrinolysis in relation to major surgery. J Cardiothorac Vasc Anesth 1997b; 11: 322-8.

Dahl O E. Thromboprophylaxis in hip arthroplasty. New frontiers and future strategy. Acta Orthop Scand 1998; 69: 339-42.

Dahl O E. Mechanisms of hypercoagulability. Thromb Haemost 1999; 82: 902-6.

Dahl O E, Johnsen H, Kierulf P, Molnar I, Ro J S, Vinje A, Mowinckel P. Intrapulmonary thrombin generation and its relation to monomethylmethacrylate plasma levels during hip arthroplasty. Acta Anaesthesiol Scand 1992; 36: 331-5.

Dahl O E, Garvik L J, Lyberg T. Toxic effects of methylmethacrylate monomer on leukocytes and endothelial cells in vitro. Acta Orthop Scand 1994; 65: 147-53.

Dahl O E, Aspelin T, Ruyter I E, Lyberg T. Evidence for induction of cardiorespiratory and vascular complications by methylmethacrylate monomer in hip replacement surgery. Laboratory and animal investigations. Hip Int 1997; 7: 158-68.

EN ISO 10993-12. Biological evaluation of medical devices - Part 12: Sample preparation and reference materials. Official Journal of European Community (1996).

Granchi D, Stea S, Ciapetti G, Savarino L, Cavedagna D, Pizzoferrato A. In vitro effects of bone cements on the cell cycle of osteoblast-like cells. Biomaterials 1995; 16: 1187-92.

Granchi D, Ciapetti G, Filippini F, Stea S, Cenni E, Pizzoferrato A, Toni A. Modulation of pro- and antiapoptotic genes in lymphocytes exposed to bone cements. J Biomater Sci Polym Ed 2000a; 11: 633-46.

Granchi D, Ciapetti G, Filippini F, Stea S, Cenni E, Pizzoferrato A, Toni A. In vitro cytokine production by mononuclear cells exposed to bone cement extracts. Biomaterials 2000b; 21: 1789-95.

Haake D A, Berkman S A. Venous thromboembolic disease after hip surgery. Risk factors, prophylaxis, and diagnosis. Clin Orthop 1989; 242: 212-31.

Hansen M B, Nielsen S E, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 1989; 119: 203-10.

Hogevold H E, Hoiseth A, Reikeras O. Deep vein thrombosis after total hip replacement. A venographic study. Acta Radiol 1990; 31: 571-3.

Hong J, Andersson J, Ekdahl K N, Elgue G, Axén N, Larsson R, Nilsson B. Titanium is a highly thrombogenic biomaterial: possible implications for osteogenesis. Thromb Haemost 1999; 82: 58-64.

Laupacis A, Rorabeck C, Bourne R, Tugwell P, Bullas R, Rankin R, Vellet A D, Feeny D, Wong C. The frequency of venous thrombosis in cemented and non-cemented hip arthroplasty. J Bone Joint Surg (Br) 1996; 78: 210-2.

Rae T. Tolerance of mouse macrophages in vitro to barium sulfate used in orthopedic bone cement. J Biomed Mater Res 1977; 11: 839-46.

Schoenfeld G J, Conard E P, Lautenschlager F W. Monomer release from methacrylate cements during simulated in vivo polymerization, J. Biomed Mater Res 1979; 13: 135-47.

Sharrock N E, Go G, Harpel P C, Ranawat C S, Sculco T P, Salvati E A. The John Charnley Award. Thrombogenesis during total hip arthroplasty. Clin Orthop 1995; 319: 16-27.