

Effects of bone cement particles on the function of pseudocapsule-derived fibroblasts

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Background Despite the wide clinical use of bone cement, little is known about cellular responses to the debris from this material. We thus investigated the effects of bone cement particles on the secretion of soluble osteotropic factors in prosthetic pseudomembrane-derived fibroblasts.

Methods Bone cement particles were added to fibroblasts maintained in tissue culture. The secretions of soluble receptor activator for nuclear factor kappa B ligand and osteoprotegerin together with interleukin-6 and tumor necrosis factor-alpha were assessed by enzyme-linked immunosorbent assays. The fibroblasts were also co-cultured with osteoclast precursors in the presence and absence of particles, and we assessed osteoclast formation and bone resorption.

Results The particles produced an increase in the secretion of soluble receptor activator for nuclear factor kappa B ligand, interleukin-6 and tumor necrosis factor-alpha, but not osteoprotegerin. At a concentration of 88 particles/cell, bone cement particles yielded a 2-fold increase (327 pg/mL) in soluble receptor activator for nuclear factor kappa B ligand secretion, a 5-fold (239 pg/mL) increase in interleukin-6 secretion and 4-fold (129 pg/mL) increase in tumor necrosis factor-alpha secretion. The particles also enhanced bone resorption in the co-culture group. Both the increase in soluble receptor activator for nuclear factor kappa B ligand secretion and the increase in bone resorption were inhibited by the addition of neutralizing antibodies to the proinflammatory cytokines.

Interpretation Our findings show that bone cement particles are capable of stimulating the secretion of soluble receptor activator for nuclear factor kappa B ligand in pseudocapsule-derived fibroblasts by increasing the

secretion of proinflammatory cytokines, and may also promote implant loosening. ■

Aseptic loosening remains the primary cause of failure in total joint arthroplasty, and the generation of aseptic loosening is partly due to the production of particulate debris that arises from the materials of implants—namely metal, polyethylene and bone cement (Gallo et al. 2002, Haynes et al. 2004). In the process of periprosthetic osteolysis, a pseudocapsular tissue of fibroblasts, macrophages and foreign-body giant cells develops surrounding the artificial joint. All types of cell of this pseudocapsule contain wear debris. Several studies (Green et al. 2000, Mabrey et al. 2002, Sabokbar et al. 2003) have analyzed debris obtained from these tissues, and reported that the most effective particles that promoted osteolysis were in the size range 0.3–10 µm. Other studies (Baumann et al. 2004, Crotti et al. 2004) have suggested that polyethylene and metal are the most common materials contributing to the production of wear debris. It was also revealed that such wear particles are able to promote the secretion of receptor activator for nuclear factor kappa B ligand (RANKL) in periprosthetic pseudocapsule and promote bone resorption. Although less is known about the biological effects of bone cement debris, several studies have shown that bone cement debris also contributes to prosthetic loosening (Abbas et al. 2003, Skoglund et al. 2003).

RANKL exists in two forms in vivo: soluble RANKL (sRANKL) and transmembrane RANKL. Osteoprotegerin (OPG) is a decoy receptor for RANKL. The ratio of RANKL/OPG plays an important role in osteoclast differentiation, activation and survival (Blair and Athanasou 2004). Proinflammatory cytokines (e.g. tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6)) and other factors regulate osteoclastogenesis and bone resorption through modulating osteoclastogenesis as well as through modulation of the expression of RANKL and OPG. It has been shown that the expression of RANKL is elevated in the tissues surrounding failed prostheses (Crotti et al. 2004), and the pseudosynovial fluid from loosened total hip prostheses has been found to contain high levels of RANKL and to be capable of inducing osteoclast formation (Mandelin et al. 2005a).

Fibroblasts are abundant in the periprosthetic pseudocapsule. Not only the fibroblasts from synovium and pseudocapsule (Sakai et al. 2002, Mandelin et al. 2005b), but also the fibroblasts from skin, spleen and respiratory diaphragm (Quinn et al. 2000) have been reported to be able to secrete RANKL and support bone resorption. Using a mouse model, Bukata et al. (2004) also proved that fibroblasts could phagocytose particles, which resulted in an increase in the secretion of proinflammatory cytokines. We therefore hypothesized that fibroblasts may play an important role in periprosthetic osteolysis, and that bone cement particles might stimulate secretion of sRANKL as well as other osteoclastogenic factors in fibroblasts, thus contributing to prosthetic osteolysis.

To investigate the effect of bone cement particles on bone resorption, we co-cultured peripheral blood monocytes (PBMCs) with fibroblasts in the presence and absence of particles, and observed the formation of osteoclasts by investigating the formation of tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells (MNCs) on coverslips and lacunar resorption pits on bone slices. To investigate the soluble factors, a cell filter was used in the co-culture of PBMCs and fibroblasts, which prevented PBMCs from coming in contact with fibroblasts and bone cement particles while allowing free passage of soluble proteins.

Material and methods

Bone cement particles

Commercially available bone cement (polymethylmethacrylate with 9.2% BaSO₄) provided by Depuy CMW Ltd., Blackpool, UK) was polymerized according to the manufacturer's instructions, and crushed to powder form. The size distribution of bone cement particles was determined by scanning electron microscopy, which demonstrated that most (> 80%) of the particles were between 1 and 10 μ m in diameter, and the density of the particles was 1.1×10^5 particles/ μ g. The particles were then weighed, suspended in culture medium, and sonicated for 10 min before being added to culture wells. The particle suspensions were regularly tested for endotoxin activity (E-Toxate; Sigma, UK) and discarded if the test results were positive.

Preparation of fibroblasts

The inner granulomatous tissue of the periprosthetic pseudocapsular tissue was harvested from 7 patients undergoing surgery on revision hips (average age 72 years, 4 men). The samples were cut into small pieces and digested in alpha-minimum essential medium (MEM) (Gibco, UK) containing 1 mg/mL collagenase type I, for 60 min at 37°C. This was followed by a further 45 min of incubation in versene (Gibco, UK). The isolated cells were suspended in MEM containing 10% fetal bovine serum (FBS) (Gibco, UK) and cultured for 3 passages prior to further experiments.

Treatment of fibroblasts with bone cement particles

Fibroblasts (5×10^4 cells per well) were added to 17-mm wells of a 24-well tissue culture plate (Nunc, UK) and cultured for 24 h to allow the cells to settle. The medium was then changed and bone cement particles of different concentrations (11, 22, 44, 88, 176 particles/cell) were added. Cells cultured only with MEM containing 10% FBS were also set up as control. All cells were cultured under static conditions. The culture was stopped 96 h after the addition of particles, and the conditioned media were collected for the measurement of sRANKL, OPG and proinflammatory cytokines. A time course experiment was also set up, in which 88 particles were added per cell and the culture was

stopped 6, 12, 24, 48 and 96 h after the addition of particles.

Measurement of sRANKL, OPG and pro-inflammatory cytokines in the conditioned medium

The conditioned media were centrifuged to remove cellular debris. The concentrations of IL-6, TNF- α , sRANKL and OPG were measured by sandwich enzyme-linked immunosorbent assays (ELISAs) following the instructions that came with the kits. The ELISA kits for IL-6 (range, 8–500 pg/mL) and TNF- α (range, 0–2,000 pg/mL) were purchased from R&D Systems (Abingdon, UK). The ELISA kits for sRANKL (range, 31.25–2,000 pg/mL) and OPG (2–100 U/L) came from Bender MedSystems GmbH (Vienna, Austria).

Isolation of PBMCs

PBMCs were isolated from the peripheral blood of 7 healthy male donors (average age 34). Whole blood was diluted 1:2 in MEM, and the monocytes were separated from whole blood by Ficoll-Hypaque (Pharmacia, UK) gradient centrifugation (693 g) for 20 min. The mononuclear cells in the interface layer were resuspended in MEM with 10% FCS and the number of cells was determined after lysis of red blood cells.

Preparation of co-cultures

Fibroblasts (5×10^4 cells per well) were seeded in the 17-mm wells of a 24-well tissue culture plate, 24 h before transfer of the PBMCs. In the “filter” group, 3.0- μ m transwell inserts (Costar, UK) were put into the 17-mm wells containing fibroblasts just before transfer of the PBMCs.

Sterile 6-mm glass coverslips and human cortical bone slices (4 mm) were placed in 7-mm wells of a 96-well tissue culture plate. PBMCs were added 24 h later at a final concentration of 2×10^5 cells per well. After 2 h of incubation, the bone slices and coverslips were removed from the 96-well plates, washed vigorously in warm MEM to remove non-adherent cells, and then transferred to the 17-mm wells or to the filter inserts (see above).

1 mL MEM/FCS with M-CSF (Macrophage Colony Stimulating Factor) (final concentration 25 ng/mL) was added to each 17-mm well. At this point, 88 particles/cell of bone particles

were added to the fibroblasts being cultured at the bottoms of the wells. RANK:Fc (a recombinant RANKL inhibitor) (final concentration 10 ng/mL; Sigma, UK) and neutralizing antibodies against IL-6 and TNF- α (final concentration 10 ng/mL; R&D Systems, Abingdon, UK) were added to the wells. As controls, two other groups were also set up where either particles or fibroblasts were not added. The cultures were stopped after 1, 14 or 21 days, with the media being replenished every 3 days.

Cytochemical assessment of osteoclast formation

After 1 and 14 seven days, coverslips were removed and stained histochemically for the osteoclast-associated enzyme TRAP using a kit from Sigma Chemicals (Pool, UK). Cells containing three or more nuclei were considered to be multinucleate cells (MNCs).

Functional assessment of osteoclast activity

After 1 and 21 days in culture, the bone slices were removed from the culture wells and (a) washed in phosphate-buffered saline, (b) treated with 1 M ammonium hydroxide overnight, (c) rinsed vigorously in distilled water, and (d) cleaned by ultrasonication. After staining with toluidine blue, the lacunar resorption pits were examined by light microscopy and the number of pits was calculated. For every sample, three bone slices were set up in each group.

Statistics

We used analysis of variance and two-tailed paired Student's t-test. The level of significance was set at $p < 0.05$.

Results

Histology of the pseudocapsular tissue

The pseudocapsular tissue from which the fibroblasts were derived consisted of macrophage-like cells, fibroblastic stromal cells and giant cells (Figure 1). Particles of metal, polyethylene and bone cement were observed in the tissue. Giant cells were usually located in the areas where wear particles existed.

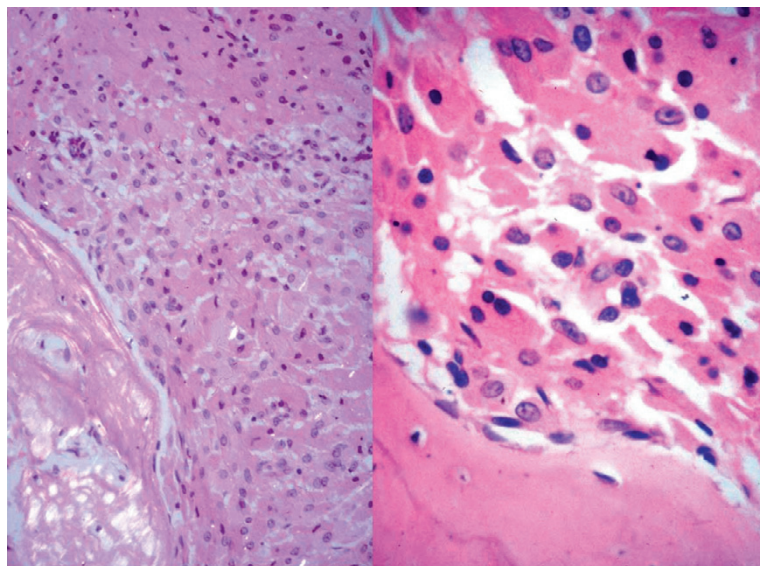


Figure 1. The pseudocapsular tissue consisting of macrophage-like cells, fibroblastic stromal cells, giant cells and wear particles.

Effect of bone cement particles on the secretion of sRANKL, OPG and cytokines

The addition of bone cement particles increased the secretion of IL-6, TNF- α and sRANKL, but not OPG. The levels of secretion increased in a dose-dependent manner, the minimum effect being at a concentration of 22 particles/cell for both IL-6 and TNF- α , and 11 particles/cell for sRANKL. The peak effects were achieved at a concentration of 88 particles/cell for all the three. Higher concentrations of particles reduced the levels of secretion, possibly due to cytotoxic effects. At 88 particles/cell, bone cement particles yielded a 5-fold increase in interleukin-6, a 3.7-fold increase in TNF- α and a 2-fold increase in sRANKL (Figure 2).

Time course experiments showed that the secretion of IL-6 and TNF- α were enhanced as early as 6 h after the addition of particles, while the increase in the secretion of sRANKL was observed only 24 h after the addition of particles (Figure 3).

The addition of either anti-IL-6 or anti-TNF- α neutralizing antibody alone did not inhibit the increase in sRANKL secretion resulting from stimulation by bone cement particles, while the addition of both types of antibodies in combination inhibited sRANKL secretion (327 (SEM 17) without antibodies vs. 237 (SEM 21) with antibodies; p

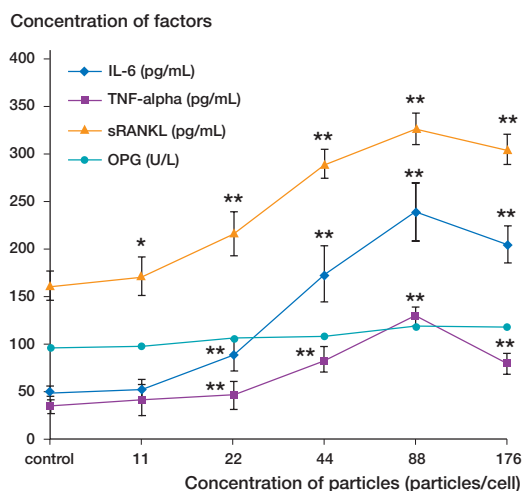


Figure 2. Effects of bone cement particles on the secretion of sRANKL, OPG, IL-6 and TNF- α in pseudocapsule-derived fibroblasts. Particles of different concentrations were added to fibroblasts and incubated for 96 h, and the conditioned media were collected for the measurement of sRANKL, IL-6 and TNF- α using ELISA. The secretion of sRANKL, IL-6 and TNF- α was increased by the addition of particles in a dose-dependent manner, with the minimum effect at a concentration of 11 particles/cell for sRANKL and 22 particles/cell for both IL-6 and TNF- α . The maximum effect was achieved at 88 particles/cell for all the three factors. n = 7. * indicates p < 0.05; ** indicates p < 0.01.

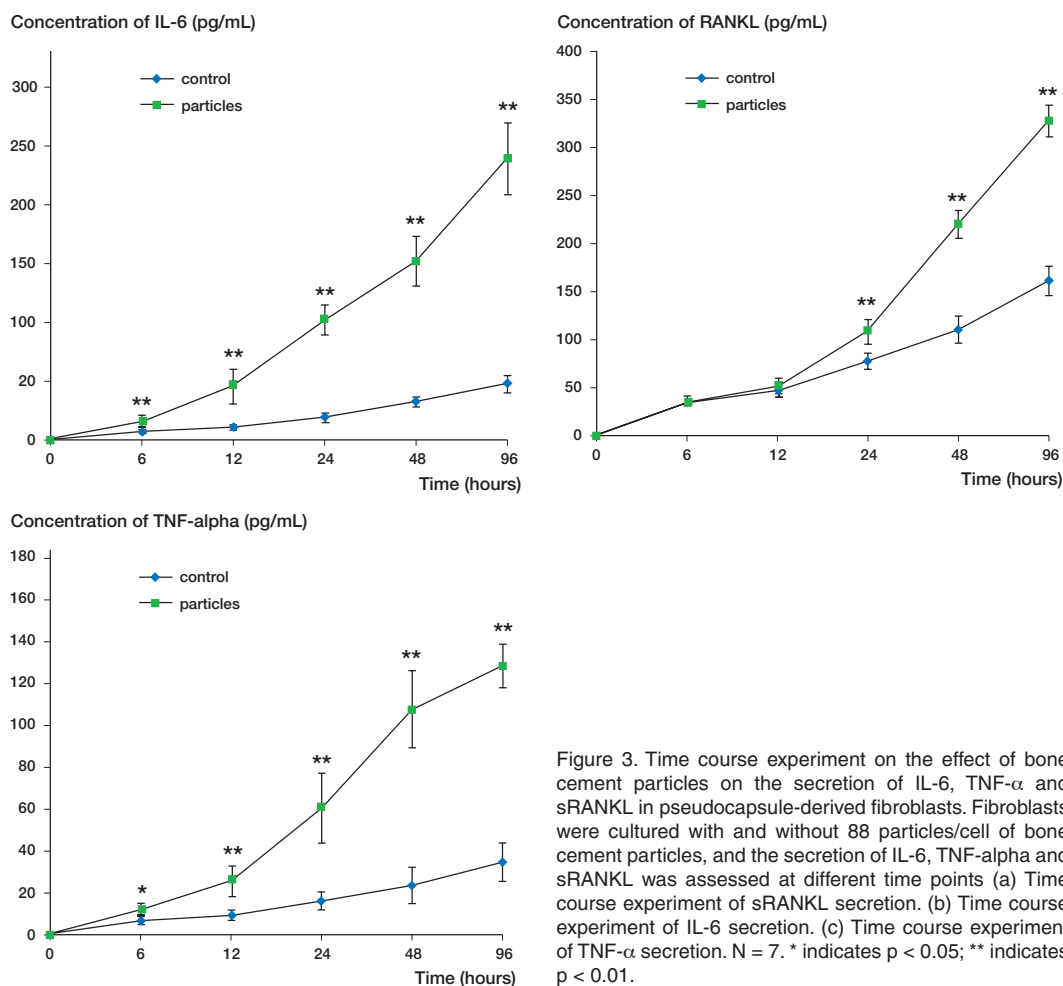


Figure 3. Time course experiment on the effect of bone cement particles on the secretion of IL-6, TNF- α and sRANKL in pseudocapsule-derived fibroblasts. Fibroblasts were cultured with and without 88 particles/cell of bone cement particles, and the secretion of IL-6, TNF- α and sRANKL was assessed at different time points (a) Time course experiment of sRANKL secretion. (b) Time course experiment of IL-6 secretion. (c) Time course experiment of TNF- α secretion. N = 7. * indicates $p < 0.05$; ** indicates $p < 0.01$.

< 0.001) and the ratio of RANKL/OPG (2.91 (SEM 0.42) without antibodies vs. 2.15 (SEM 0.32) with antibodies; $p < 0.01$) significantly (Table 1).

Osteoclast formation and activity.

Neither TRAP-positive MNCs nor lacunar resorption pits were observed in any of the groups after 1 day of incubation, which meant that at the beginning there were no osteoclasts in the cell culture. After 14 days, TRAP-positive MNCs were observed in all of the co-culture groups (Figure 4a), but not in the group where fibroblasts were not added.

After 21 days of incubation, various numbers of lacunar resorption pits on bone slices were found in the co-culture groups (Figure 4b), while no lacunar pits were observed in the absence of fibroblasts.

Lacunar resorption was also observed in the filter group, even though the number of pits was fewer than that of the co-culture group with direct contact. Bone cement particles increased the number of lacunar pits both in the co-culture group with direct contact and the cell filter groups. The addition of RANK:Fc completely blocked the formation of lacunar pits (Table 2). Neither neutralizing anti-IL-6 antibody nor anti-TNF- α antibody blocked the enhancing effect of bone cement on bone resorption when they were added separately, while the addition of anti-IL-6 antibody together with anti-TNF- α antibody inhibited this process significantly (Table 2).

Table 1. The effect of neutralizing antibodies against TNF- α and IL-6 on sRANKL/OPG secretion induced by bone cement particles in pseudomembrane-derived fibroblasts. Values (N = 7) are mean (SEM)

Group	Conditions	Concentration of sRANKL (pg/mL)	Concentration of OPG (U/mL)	Ratio of sRANKL/OPG
A	No particles	161 (15)	96 (9)	1.68 (0.12)
B	Particles alone	327 (17) ^a	114 (19)	2.91 (0.42) ^a
C	Particles + anti-TNF- α	314 (25)	121 (23)	2.66 (0.45)
D	Particles + anti-IL-6	3112 (17)	102 (14)	3.11 (0.46)
E	Particles + anti-TNF- α + anti-IL-6	237 (21) ^b	112 (15)	2.15 (0.32) ^b

^a indicates that $p < 0.01$, vs. A
^b indicates that $p < 0.01$, vs. B

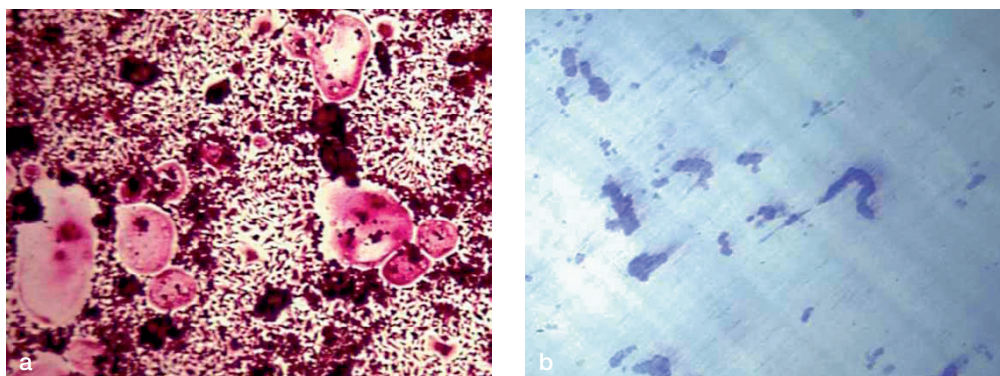


Figure 4. The formation of TRAP-positive MNCs and lacunar resorption pits. PBMCs and fibroblastic stromal cells were co-cultured for 14 and 21 days, and TRAP-positive multinucleated cells (a) and lacunar resorption pits (b) were counted.

Table 2. The effect of bone cement particles on fibroblast-supported bone resorption. Values (N = 7) are mean (SEM)

Group	Conditions	Number of pits
A	PBMCs + particles	0
B	Co-culture	52 (14)
C	Co-culture + particles	86 (11) ^a
D	Co-culture + particles + RANK:Fc	0
E	Co-culture + filter	19 (5.2) ^b
F	Co-culture + filter + particles	24 (6.7) ^c
G	Co-culture + filter + particles + anti-IL-6	23 (7.0)
H	Co-culture + filter + particles + anti-TNF-alpha	24 (6.6)
I	Co-culture + filter + particles + anti-IL-6 + anti-TNF-alpha	19 (5.5) ^d

^a $p < 0.001$, vs B
^b $p < 0.001$, vs B
^c $p = 0.001$, vs E
^d $p = 0.008$, vs F

Discussion

Our findings show that bone cement particles

are capable of increasing the secretion of soluble osteoclastogenic factors in pseudocapsule-derived stromal cells, and of supporting osteoclastogen-

esis and bone resorption in the presence of M-CSF without the addition of any other osteoclastogenic factors. Bone cement particles promoted sRANKL expression at least partly through the secretion of IL-6 and TNF- α .

RANKL stimulates osteoclast differentiation and activity through interaction with RANK expressed in osteoclast precursors and mature osteoclasts. Also, a decoy receptor of RANKL, namely OPG, inhibits bone resorption through competition with RANK in binding to RANKL. Most factors affect bone turnover through modulation of the ratio of RANKL/OPG (Blair and Athanasou 2004). Increased secretion of RANKL in periprosthetic tissue has been demonstrated in response to the stimulation of debris (Crotti et al. 2004). However, the authors did not mention which kind of debris (for example metal particles, bone cement particles and PZ particles) stimulated RANK secretion. In our study, the presence of bone particles increased the secretion of IL-6 and TNF- α , which was followed by an increase in sRANKL secretion while the secretion of OPG was not increased significantly.

IL-6 and TNF- α have multiple roles related to the metabolism of bone. Besides their synergetic effects (with RANKL) on osteoclastogenesis (Fuller et al. 2002, Liu et al. 2005), IL-6 and TNF- α have also been reported to be capable of supporting the differentiation and activity of osteoclasts in a RANKL-independent manner (Kobayashi et al. 2000, Kudo et al. 2003). As proinflammatory cytokines, IL-6 and TNF- α also affect bone resorption through modulating the expression of RANKL and OPG (Palmqvist et al. 2002, Kwan et al. 2004). So even small changes in the secretion of IL-6 and TNF- α could markedly affect bone resorption. The expression of IL-6 and TNF- α have been reported to be increased in periprosthetic tissues (Campbell et al. 2002). In our study, the secretion of IL-6 and TNF- α in pseudocapsule-derived fibroblasts was increased by the stimulation from bone cement particles and was followed by an increase in sRANKL secretion. This increased sRANKL secretion was blocked by the addition of neutralizing antibody against IL-6 and TNF- α . Thus, we believe that bone cement particles stimulated sRANKL secretion at least partly through the increase in secretion of IL-6 and TNF- α .

Previous studies have also reported that fibroblasts are capable of phagocytosing wear debris and secreting cytokines and RANKL to support osteoclast formation and bone resorption (Bukata et al. 2004, Horiki et al. 2004). Osteoclastogenesis supported by other sources of fibroblasts happened only in the presence of osteoclastogenic factors other than just M-CSF (such as dexamethasone), while pseudomembrane-derived fibroblasts were able to support osteoclastogenesis and bone resorption only in the presence of M-CSF. At the same time, we found that pseudocapsule-derived fibroblasts were capable of secreting soluble osteoclastogenic factors, including proinflammatory cytokines and sRANKL, which were enough to support bone resorption in the presence of M-CSF. The soluble factors produced by pseudocapsular tissue are transported by joint fluid to bone tissue where osteoclasts resorb bone. On the other hand, pseudocapsular tissue-derived fibroblasts also produce transmembrane RANKL, through which they stimulate osteoclast precursors and osteoclasts, and the primed osteoclast precursors and osteoclasts can then migrate to bone tissue and develop into mature osteoclasts and resorb bone. Since the osteoclast-mediated bone resorption in the process of aseptic loosening takes place in the interface between bone cement and bone—which is out of the reach of pseudocapsule—the soluble factors seem to play a more important role than that of the membrane-binding ones in the process pseudocapsular tissue-supported bone resorption. The formation of lacunar resorption pits was completely blocked by the addition of RANK:Fc, but not by anti-TNF- α neutralizing antibody, which suggests that pseudocapsule-derived fibroblasts support osteoclast-mediated bone resorption through a RANKL/RANK-related mechanism. Consistent with the increase in sRANKL levels, the formation of lacunar resorption was increased by the addition of bone cement particles. The addition of neutralizing anti-IL-6 antibody together with anti-TNF- α antibody inhibited not only the secretion of sRANKL but also the formation of lacunar pits. We believe that besides blocking the effect of IL-6 and TNF- α on osteoclasts, the neutralizing antibodies also blocked the effect of cytokines on fibroblasts—which in turn inhibited the increase in sRANKL secretion and led to the decrease in bone resorption.

The generation of periprosthetic osteolysis is complex, involving several types of cells and humeral factors. Our study suggests that bone cement particles are capable of enhancing bone resorption supported by periprosthetic pseudocapsule-derived fibroblasts, and that stromal cells react to bone cement particles by increasing the secretion of sRANKL, IL-6 and TNF- α . Through expression of RANKL, IL-6 and TNF- α , the pseudocapsule-derived fibroblasts supported osteoclastogenesis, which was blocked by RANK:Fc and was inhibited by neutralizing antibodies against IL-6 and TNF- α . Based on these findings, RANK:Fc, IL-6 antagonist and TNF- α antagonist may have the therapeutic potential to prevent or treat aseptic loosening.

Author contributions

QYF and SGS designed the research, wrote the paper and collected and analyzed data. SGS, BAM, YZ and MHZ performed the research.

No competing interests declared.

- Abbas S, Clohisy J C, Abu-Amer Y. Mitogen-activated protein (MAP) kinases mediate PMMA-induction of osteoclasts. *J Orthop Res* 2003; 21 (6): 1041-8.
- Baumann B, Rader C P, Seufert J, Noth U, Rolf O, Eulert J, Jakob F. Effects of polyethylene and TiAlV wear particles on expression of RANK, RANKL and OPG mRNA. *Acta Orthop Scand* 2004; 75 (3): 295-302.
- Blair H C, Athanasou N A. Recent advances in osteoclast biology and pathological bone resorption. *Histol Histopathol* 2004; 19 (1): 189-99.
- Bukata S V, Gelinas J, Wei X, Rosier R N, Puzas J E, Zhang X, Schwarz E M, Song X Y, Griswold D E, O'Keefe R J. PGE2 and IL-6 production by fibroblasts in response to titanium wear debris particles is mediated through a Cox-2 dependent pathway. *J Orthop Res* 2004; 22 (1): 6-12.
- Campbell P A, Wang M, Amstutz H C, Goodman S B. Positive cytokine production in failed metal-on-metal total hip replacements. *Acta Orthop Scand* 2002; 73 (5): 506-512.
- Crotti T N, Smith M D, Findlay D M, Zreiqat H, Ahern M J, Weedon H, Hatzinikolous G, Capone M, Holding C, Haynes D R. Factors regulating osteoclast formation in human tissues adjacent to peri-implant bone loss: expression of receptor activator NF-kappaB, RANK ligand and osteoprotegerin. *Biomaterials* 2004; 25 (4): 565-73.
- Fuller K, Murphy C, Kirstein B, Fox S W, Chambers T J. TNF-alpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. *Endocrinology* 2002; 143 (3): 1108-18.
- Gallo J, Kaminek P, Ticha V, Rihakova P, Ditmar R. Particle disease. A comprehensive theory of periprosthetic osteolysis: a review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2002; 146 (2): 21-8.
- Green T R, Fisher J, Matthews J B, Stone M H, Ingham E. Effect of size and dose on bone resorption activity of macrophages by in vitro clinically relevant ultra high molecular weight polyethylene particles. *J Biomed Mater Res* 2000; 53 (5): 490-7.
- Haynes D R, Crotti T N, Zreiqat H. Regulation of osteoclast activity in peri-implant tissues. *Biomaterials* 2004; 25 (20): 4877-85.
- Horiki M, Nakase T, Myoui A, Sugano N, Nishii T, Tomita T, Miyaji T, Yoshikawa H. Localization of RANKL in osteolytic tissue around a loosened joint prosthesis. *J Bone Miner Metab* 2004; 22 (4): 346-51.
- Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinoshita M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin T J, Suda T. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J Exp Med* 2000; 191 (2): 275-86.
- Kudo O, Sabokbar A, Pocock A, Itonaga I, Fujikawa Y, Athanasou N A. Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. *Bone* 2003; 32 (1): 1-7.
- Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine Growth Factor Rev* 2004; 15 (1): 49-60.
- Liu X H, Kirschenbaum A, Yao S, Levine A C. Cross-talk between the IL-6 and prostaglandin E2 signaling systems results in enhancement of osteoclastogenesis through effects on the OPG/RANKL/RANK system. *Endocrinology* 2005; 146 (4): 1991-8.
- Mabrey J D, Afsar-Keshmiri A, Engh G A, Sychterz C J, Wirth M A, Rockwood C A, Agrawal C M. Standardized analysis of UHMWPE wear particles from failed total joint arthroplasties. *J Biomed Mater Res* 2002; 63 (5): 475-83.
- Mandelin J, Liljestrom M, Li T F, Ainola M, Hukkanen M, Salo J, Santavirta S, Konttinen Y T. Pseudosynovial fluid from loosened total hip prosthesis induces osteoclast formation. *J Biomed Mater Res B Appl Biomater* 2005a; 74 (1): 582-8.
- Mandelin J, Li T F, Hukkanen M, Liljestrom M, Salo J, Santavirta S, Konttinen Y T. Interface tissue fibroblasts from loose total hip replacement prosthesis produce receptor activator of nuclear factor-kappaB ligand, osteoprotegerin, and cathepsin K. *J Rheumatol* 2005b; 32 (4): 713-20.
- Palmqvist P, Persson E, Conaway H H, Lerner U H. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand, osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae. *J Immunol* 2002; 169 (6): 3353-62.
- Quinn J M, Horwood N J, Elliott J, Gillespie M T, Martin T J. Fibroblastic stromal cells express receptor activator of NF-kappa B ligand and support osteoclast differentiation. *J Bone Miner Res* 2000; 15 (8): 1459-66.

- Sabokbar A, Pandey R, Athanasou N A. The effect of particle size and electrical charge on macrophage-osteoclast differentiation and bone resorption. *J Mater Sci Mater Med* 2003; 14 (9): 731-8.
- Sakai H, Jingushi S, Shuto T, Urabe K, Ikenoue T, Okazaki K, Kukita T, Kukita A. Fibroblasts from the inner granulation tissue of the pseudocapsule in hips at revision arthroplasty induce osteoclast differentiation, as do stromal cells. *Ann Rheum Dis* 2002; 61 (2): 103-9.
- Skoglund B, Aspenberg P. PMMA particles and pressure--a study of the osteolytic properties of two agents proposed to cause prosthetic loosening. *J Orthop Res* 2003; 21 (2): 196-201.