Effect of particles and interface conditions on fibrous tissue interposition between bone and implant. A particle challenge model in rabbit

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Interposed fibrous tissue at bone–implant interfaces was quantitatively measured in the presence or absence of polyethylene (PE) or alumina particles. Three different conditions of the interface were designed by implanting a pre-polymerized polymethylmethacrylate (PMMA) plug (plug group), a doughy PMMA (injection group) and a hydroxyapatite (HA) plug (HA group) in the hole drilled at the intercondylar notch of rabbit knees. PE ($170 \pm 18\,\mu m$) or alumina particles ($88 \pm 26\,\mu m$) were repeatedly administered into the knee joints at one month intervals (six times). All animals were sacrificed seven months after the implantation. The bone–implant interface was histomorphometrically examined using undecalcified ground sections. In the plug group, the PE particles significantly increased the extent of the interposed fibrous tissue ($p < 0.05$), while the alumina particles showed no effect. In contrast, both particles showed no significant effects in the injection and the HA groups. These results indicate that both particle characteristics and conditions of the bone–implant interface affected particle-induced fibrous tissue interposition. The loose PMMA plug with PE particles induced the greatest amount of fibrous tissue interposition.

1. Introduction

In total joint replacements, periprosthetic bone loss (osteolysis) may be one of the most important causes of late failure. Macro- and microscopic examinations of failed total hip prostheses have described the presence of fibrous tissue and wear debris with macrophages and foreign body giant cells at the bone–implant interface [1–7]. Various particulate materials, including polyethylene (PE), polymethylmethacrylate (PMMA) and metals, have been cited as the underlying cause of the consecutive osteolysis [7–10]. The histological reaction to these particles has been investigated [11–13].

Recently, PE particles are considered to play a major role in osteolysis. It is reported that PE particles are phagocytosed by macrophages, and these cells in turn release inflammatory mediators that stimulate osteoclastic bone resorption [7, 13–17]. Alumina–alumina combination is one of the solutions to avoid the production of PE particles. The reported clinical results of alumina–alumina total hip replacement were excellent [18]. It is not well known whether these alumina particles induce bone resorption or not.

Since these events occur at the bone–implant interface, efforts to strengthen the resistance of the interface to particle migration have been intended. The improvement of cementing techniques and the use of HA-coated prostheses have reduced the incidence of osteolysis [19, 20]. However, the exact relationship between the condition of the interface and the progression of osteolysis is still unclear.

Howie *et al.* [21] investigated bone resorption at the bone–PMMA interface in a rat model. In the presence of PE particles, a cellular connective tissue layer was found around pre-polymerized PMMA plugs. However, the results have not been analyzed quantitatively. We have therefore histomorphometrically analyzed the interposed fibrous tissue at the bone–PMMA interface and our preliminary experiment showed that the PE particles tended to increase the amount of interposed fibrous tissue [22].

The purpose of the current study was to compare quantitatively the extent of interposed fibrous tissue after administration of PE or alumina particles. Three groups were designed to simulate three different conditions of the bone–implant interface, i.e. a loose cemented prosthesis, a well-fixed cemented prosthesis and a HA-coated prosthesis. Also to simulate the clinical situation in which wear particles are continuously produced, the particles were repeatedly administered.

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2. Materials and methods

2.1. Operative techniques

Nineteen male Japanese white rabbits, weighing approximately 3 kg, were used. General anaesthesia was induced and was maintained by intravenous injection of sodium pentobarbital (Nembutal®, 25 mg kg^{-1} body weight) and intramuscular injection of ketamine (Ketalar®, 5 mg kg^{-1} body weight). Both legs were shaved, cleaned and disinfected. Both knee joints were exposed through the medial parapatellar approach. A hole, 3.8 mm in diameter and 10 mm in depth, was drilled at the intercondylar notch of the femur, parallel to the shaft with a stainless-steel drill bit. The cavity was irrigated with sterile saline solution and haematostasis was confirmed after packing with gauze. Three different conditions of bone–implant interface were designed and the materials were randomly implanted.

2.1.1. PMMA plug group (plug group) ($n = 12$ knees)

PMMA (Surgical Simplex-P®, Howmedica) was injected into a Nélaton's catheter with an inner diameter of 3.5 mm. After polymerization, the rod was sectioned into cylinders (3.5 mm $\phi \times 10$ mm). These plugs were loosely inserted into a slightly larger drilled hole. The top of the implant was adjusted not to protrude from the articular surface, in order to prevent mechanical stress by the patellar or the tibial plateau.

2.1.2. PMMA injection group (injection group) ($n = 18$)

Doughy PMMA (Surgical Simplex-P®) was injected with pressure into the drilled hole using a small syringe. Overflowed PMMA was carefully removed until the surface of the PMMA was slightly lower than the articular surface.

2.1.3. Hydroxyapatite plug group (HA group) ($n = 8$)

Porous hydroxyapatite plugs (3.5 mm $\phi \times 10$ mm, Sumitomo Pharmaceutical Co., Japan) were inserted. The pore size was 50–300 μm, and the porosity was 41.6%.

2.2. Particles and administration

The PE particles used in this study were raw material PE components, and the alumina particles were fabricated using the spray dry method. The size of PE and alumina particles was measured using scanning electron microscopy (SEM). The average diameter of PE particles was 170 ± 18 μm (mean $\pm$ standard deviation) and that of alumina particles was 88 ± 26 μm.

The PE particles were administered in six rabbits, and the alumina particles were administered in four rabbits. In the rest of the nine rabbits, no particles were challenged. The distribution of particle administered knee joints is shown in Table I. In order to administer the particles, both knee joints were exposed with capsulotomy under general anaesthesia. Fifty milligrams of PE particles ($\cong 2.1 \times 10^4$ particles) or 50 mg of alumina particles ($\cong 3.6 \times 10^4$ particles) were directly sprinkled over the intercondylar notch of the knee joints. This procedure started one month after implantation and was repeated once a month for six months.

2.3. Histomorphometry

One month after the last administration, all animals were sacrificed. The distal part of the femur was harvested and was fixed in 10% neutral buffered formalin, then was dehydrated and was embedded in PMMA. Undecalcified specimens were cut sagittally with a diamond saw through the center of the drilled hole, parallel to the drilling axis, and were ground down to 50 μm.

TABLE I Percentage[a] of interposed fibrous tissue for the total length of the interface

	Particle(−)	PE	Alumina	Fisher's PLSD *post hoc* criteria		
				(−) versus PE	(−) versus Al	PE versus Al
Plug	50.9 ± 7.8 ($n = 4$)	72.8 ± 4.4 ($n = 4$)	41.4 ± 12.1 ($n = 4$)	S[c]	NS	S
Injection	15.1 ± 4.6 ($n = 10$)	14.7 ± 2.2 ($n = 4$)	18.4 ± 5.8 ($n = 4$)	NS[d]	NS	NS
HA	6.9 ± 3.5 ($n = 4$)	2.2 ± 0.8 ($n = 4$)	*NE*[b]	NS		
	Fisher's PLSD *post hoc* criteria					
Plug versus injection	S	S	S			
Plug versus HA	S	S				
Injection versus HA	NS	NS				

[a]Values are mean $\pm$ standard deviation.
[b]NE, not examined in this study.
[c]S, significant.
[d]NS, not significant.

The sections were stained with toluidine blue. With light microscopy, the distribution of the fibrous tissue at the bone–implant interface was investigated. To compare the amount of interposed fibrous tissue, the total length of the bone–implant interface and that of interposed fibrous tissue was measured (Fig. 1). The results were expressed as the percentage of the length of interposed fibrous tissue for the total length of the interface.

Multiple group comparisons were performed by using ANOVA and the Fisher's PLSD *post hoc* criteria. P values of < 0.05 were considered significant.

3. Results

Without particles, the gap between the bone and the PMMA plug was filled with thin fibrous tissue and newly formed bone (Fig. 2). By injecting PMMA with pressure, the PMMA entered into the cancellous structure of the bone, thus the interface was intricate (Fig. 3). The PMMA existed next to the bone, while fibrous tissue was partially interposed at the interface. In contrast, marked new bone formation was obvious around the HA plug and also extended into the superficial pores. Interposed fibrous tissue was scarcely observed (Fig. 4).

With PE particles, mild synovitis was observed at the time of sacrifice. Microscopically, the PE particles, which were surrounded by macrophages and foreign body giant cells, induced fibrous tissue proliferation in the synovium. In some specimens of the plug group, the bone at the interface near the aggregated PE particles underwent resorption (Fig. 5). In contrast with PE particles, the alumina particles induced smaller amounts of fibrous tissue.

The amount of interposed fibrous tissue was histomorphometrically evaluated (Table I). Without particles, the percentage of interposed fibrous tissue was significantly high in the plug group. With PE particles, the percentage significantly increased only in the plug group, while the increase was not observed in the injection and HA groups. In all groups, the alumina particles did not affect the proliferation of interposed fibrous tissue.

4. Discussion

It is reported that the periprosthetic bone loss of both linear (diffuse) pattern and lytic (localized) pattern had fibrous tissue with macrophages and foreign body giant cells [6]. These differences of radiographic appearance were not related to the histological appearance. It is considered that linear bone resorption has the possibility of developing a lytic pattern, if the fibrous tissue was exposed to a great amount of PE particles. In the present study, interposed fibrous tissue expressed a linear pattern, and the lytic pattern was not observed. It is supposed that the number of particles was not sufficient or that their size was not small enough to produce the lytic pattern.

Recently submicrometer particles were detected using SEM [23–27] and the submicroscopic PE particles are assumed to be the major culprit [19]. Various sizes of particles have been used to investigate the effects of the particles *in vivo* and *in vitro* [21, 22, 28–30], while the relation between particle size and its adverse effects was still unclear. We observed that the amount of interposed fibrous tissue increased after administration of PE particles. The size of the particles, used in this study, was larger than that reported from clinical studies [31, 32]. If it is true that submicrometer particles play a greater role in osteolysis, our results will be expected to be amplified.

Schmalzried *et al.* [6] proposed the concept of effective joint space, which is accessible to joint fluid and thus accessible to particulate debris. It is supposed that the amount of pre-existing fibrous tissue interposition at the bone–implant interface affects the particle induced osteolysis. From our histomorphometrical results, the percentage of interposed fibrous tissue was

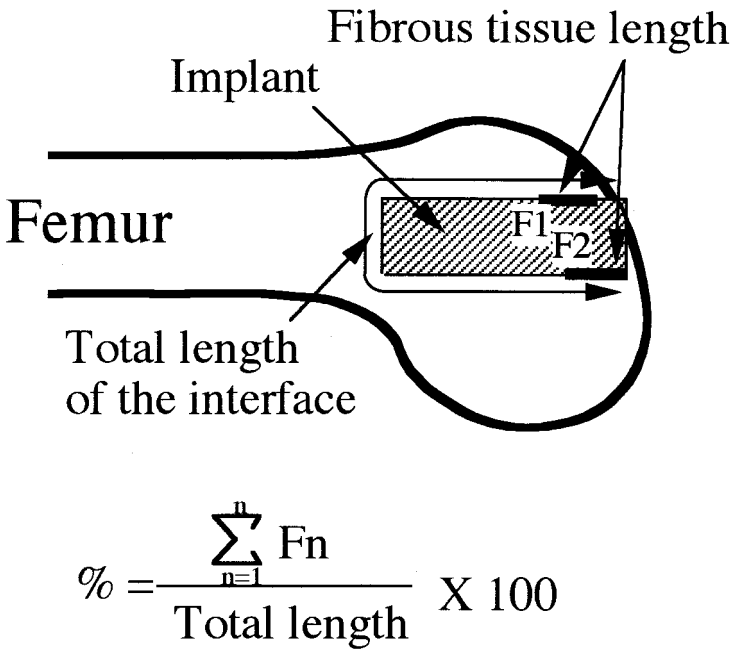

Figure 1 Schematic diagram of histomorphometry of the bone–implant interface. The total length of the bone–implant interface and that of interposed fibrous tissue (F1, F2, . . .) was measured. The percentage of the interposed fibrous tissue length for the total length was calculated.

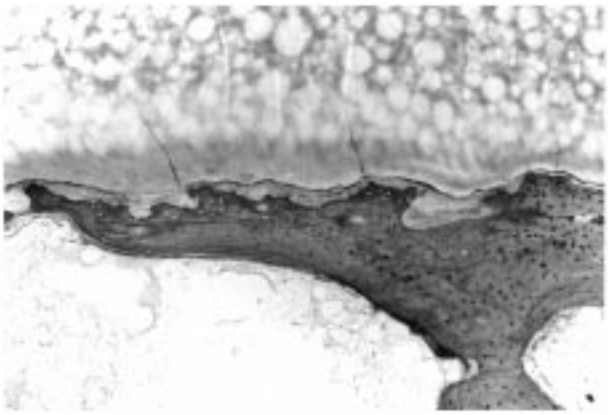

Figure 2 Photomicrograph of the bone–PMMA plug interface without particles. The upper space is occupied by the PMMA plug and the interface is interposed by thin fibrous tissue (toluidine blue, × 200).

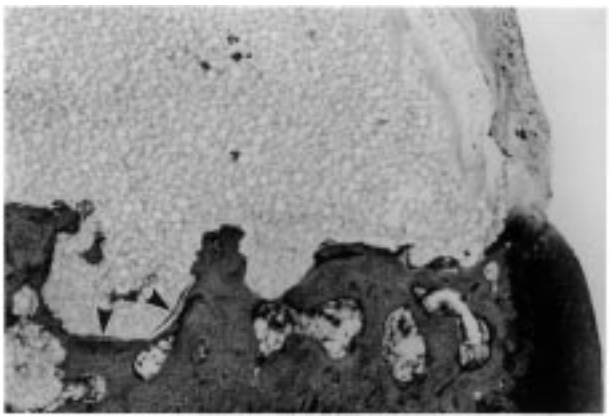

Figure 3 Photomicrograph of the bone–injected PMMA interface without particles. The articular surface is on the right, and the upper space is occupied by the PMMA. The PMMA entered into the cancellous structure of the bone. Interposed fibrous tissue is also observed (arrow heads). (Toluidine blue, × 80).

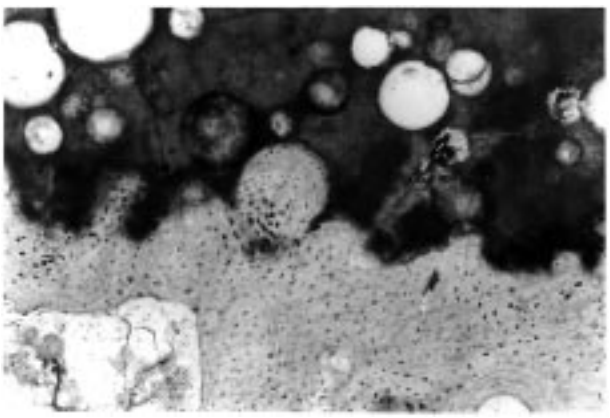

Figure 4 Photomicrograph of the bone–HA plug interface without particles. New bone formation was seen around the implant and in the superficial pores (toluidine blue, × 200).

the highest in the plug group and was the lowest in the HA group. When considering that the plug group corresponds to a loose cemented prosthesis, the injection group corresponds to a well-fixed cemented prosthesis, and the HA group corresponds to a HA-coated prosthesis, our findings are similar to those reported from clinical retrieval studies [3, 5, 6, 14].

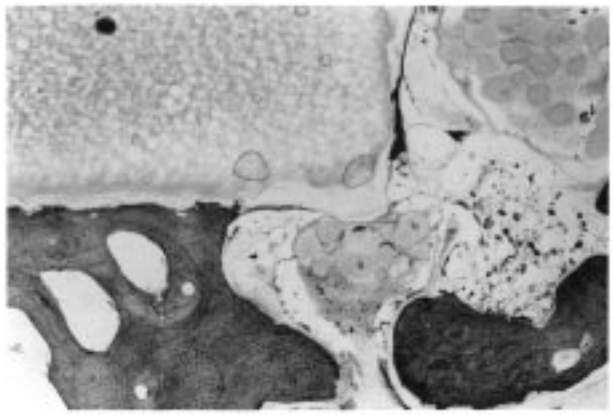

Figure 5 Photomicrograph of the bone–PMMA plug interface with PE particles. The articular surface is on the right. The PE particles were surrounded by fibrous tissue containing macrophages. The bone near the aggregated PE particles suffered resorption (toluidine blue, × 80).

Clinically, improved cementing techniques were reported to decrease the incidence of femoral stem loosening markedly [19]. It was supposed that this interface resists not only mechanical stress but also particle induced osteolysis by reducing the effective joint space. It was also reported that any calcar cavitation from the accumulation of PE wear particles tended to remain confined to that region by using HA-coated stems, since the HA coating provides an excellent ''seal'' between the implant and the host bone [20]. From our results, PE particles increased the fibrous tissue interposition in the plug group, while the particles did not reveal any effect in the injection and HA groups in which the amount of interposed fibrous tissue was smaller.

Compared with the PE particles, the alumina particles showed no effect on fibrous tissue interposition both in the plug and injection groups. We did not make the HA group with alumina particles. Since the alumina particles did not affect fibrous tissue interposition in the other two groups, it was supposed that the alumina particles would not play a role in the HA group, in which pre-existing interposed fibrous tissue was the least. The PE and the alumina particles used in this study were different in chemical nature, size and shape, thus it cannot be concluded which factors are related to the difference in our results. It is at least concluded that the alumina particles used in this study presented lower risk for osteolysis compared with the PE particles.

5. Conclusions

These results indicate that both particle characteristics and conditions of the bone–implant interface affect particle-induced osteolysis. Loose cementing with PE particles induces the greatest amount of fibrous tissue at the interface. It is proposed that fixation of the prosthesis is important in preventing osteolysis as well as improvement of the bearing couple.

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