A controlled experimental model of revision implants

Part II. Implementation with loaded titanium implants and bone graft

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ABSTRACT – We used an experimental model producing an aggressive tissue response associated with implant loosening in humans: a 6 mm polymethylmethacrylate (PMMA) cylinder was pistoning 500 μm concentrically in a 7.5 mm hole, with polyethylene (PE) particles, for 8 weeks. At 8 weeks, the PMMA implant was revised with a titanium alloy (Ti) implant, and an identical primary Ti implant was inserted contralaterally for 4 weeks. With this protocol, we evaluated primary and revision plasma-sprayed Ti implants which were loaded under stable conditions with or without allograft, or under unstable conditions without allograft (bilateral primary and revision implants, n 8 per group, 48 implants in 24 dogs). Revision implants had lower interfacial shear strength, less bone in contact with and adjacent to the implant, and resulted in higher levels of IL-6β and TNFα and lower levels of TGFβ. In both the revision and primary settings, allograft increased shear strength, stiffness and energy, bone-implant contact, and bone area adjacent to the implant. Unstable implants could not generate a mechanically sound interface, and further exacerbated the difference between primary and revision. We conclude that factors important for improving the fixation of revision implants were bone graft and a stable interface.

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To study methods that improve outcomes of revision joint replacement, we developed a controlled experimental model of the revision joint replacement setting (Part I). Here, in Part II, we implement this revision protocol to evaluate mechanical and histomorphometric differences in its primary and revision settings, and evaluate the use of bone graft in improving the interface in revision implants. In particular, we studied the following three hypotheses using titanium (Ti) implants:

1. Compared to primary implants, revision implants have (a) lower interfacial shear strength, stiffness and energy, (b) less bone adjacent to the implant, (c) less bone in contact with the implant, (d) higher levels of IL-6β and (e) TNFα (pro-inflammatory cytokines) and (f) lower ones of TGFβ (anabolic cytokine).

2. Bone graft increases shear strength, bone area, and TGFβ in the joint fluid.

3. An unstable interface exacerbates the difference between primary and revision, using these outcome measures.

Methods

The revision protocol establishing conditions like those of an aseptically-loosened cemented implant are described in Part I (Figure 1; Bechtold 2001, pp 642–9). Here in Part II, we studied the response to primary and revision plasma-sprayed titanium (Ti) implants under stable and unstable conditions, with and without bone graft. The revision protocol was used in 48 implants (24 dogs, Table 1). All experiments received approval from our institution's Animal Care and Use Committee.

General description of implant

The micromotion device consists of a 6.0 mm plasma-sprayed titanium implant pistoning 0.5 mm

Table 1. Treatment groups for loaded Ti implants (48 implants in 24 dogs; primary and revision implants were bilateral)

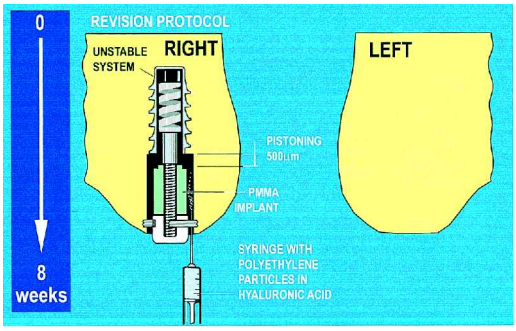
Loaded Ti implants	Stable		Stable + graft		Unstable	
	Primary	Revision	Primary	Revision	Primary	Revision
Number of implants	8	8	8	8	8	8

concentrically in a hole with a 0.75 mm gap (details in Part I). The implant was loaded in the medial femoral condyles (Figure 1) for 8 weeks in the presence of PE (0.5–50 μm; 5×10^7; 85% < 12 μm).

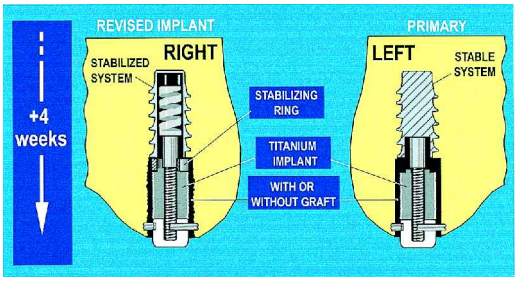
Revision setting

The revision protocol was implemented at 8 weeks by revising the PMMA implant in the right knee (Figure 1). At this operation, the cavity is scraped and lavaged, and a 6 mm plasma-sprayed titanium (Ti) implant was reinserted into the revision cavity.

Figure 1. Study design.



a. The revision protocol includes a PMMA implant pistoning in the right knee for 8 weeks in the presence of PE particles suspended in hyaluronic acid.



b. After 8 weeks, revised Ti implants are inserted into the cleaned and lavaged sclerotic cavity, and primary Ti implants are inserted into the newly drilled cavity in the contralateral left knee. Both primary and revised implants are followed for 4 weeks.

We divided the 3 revision treatment groups as follows:

Stable loaded revised implant conditions were created by placing an interference ring to stabilize the previously unstable piston (and thereby prevent further motion). The PMMA implant is revised with a Ti implant.

Stable loaded, grafted revised implant conditions were created by packing morselized allograft into the gap surrounding a Ti implant that was stabilized with an interference ring.

Unstable loaded revised implant conditions were created by revising the PMMA implant with a Ti implant, while maintaining the 500 μm of motion.

Primary setting

At the same operative setting as the revision surgery, an identical Ti implant was inserted into a newly drilled primary cavity in the contralateral left knee (Figure 1). As for the revision implants, the primary implants are stable without graft, stable with graft, or unstable.

4 weeks after the second operation, a necropsy was done and the tissue response was evaluated.

Allograft. In grafted treatment groups, allograft was prepared under sterile conditions from canine humeral heads immediately after killing for other studies. All cartilage was removed from the articulating surface, and a bone mill (fine setting, 3M, St. Paul, MN) made fine chips. The allograft was crushed further by a rongeur. The minced allograft was divided into 0.25 gm portions, which were individually frozen at –80 °C. Allograft was thawed for 1 hour before use.

Surgical procedure

The surgical procedure is described in Part I. There were no infections, and all dogs were fully bearing weight within 5 days of their second operation to insert the primary and revision Ti implants.

Outcome measures

Anteroposterior and lateral radiographs were obtained at killing, the knees were surgically exposed, and joint fluid as well as bacterial cultures taken from the joint. Joint fluid was also obtained from unoperated shoulder joints, as a control, for cytokine studies. The distal femur was removed and frozen at –20 °C.

Interfacial shear strength (pushout). Before fixation with Bouin's fluid, the implant and surrounding bone were cut into two pieces perpendicular to the implant, using a water-cooled diamond band saw (EXAKT, Norderstadt, Germany).

One of these pieces was kept frozen at –20 °C, and thawed before pushout testing. The pushout tests were done with a universal test machine (Instron Ltd.). The specimens were placed on a metal platform with a central circular opening supporting the bone to within 500 μm of the interface. A metal rod was put in the upper holding device for the axial pushout test of the implant from the surrounding tissues. A displacement rate of 5 mm/min was used for all tests and load-deformation curves were obtained. Ultimate shear strength, and apparent shear stiffness were estimated from the load-displacement curves (Søballe et al. 1992a).

Cytokines. Cytokine levels were measured in the joint fluid and in the unoperated shoulders. Levels of IL-6, TNF-α, and TGF-β were quantified, using ELISA assays (R&D Systems, Inc., Minneapolis, MN), to detect the presence of the proteins. ELISA results were corroborated by immunohistochemical staining of the retrieved interface membranes.

Quantitative histomorphometry. One transverse section was used to prepare ground sections to identify patterns of bone distribution in the former gap and/or drill hole. The ground sections were prepared by sectioning transversely to the long axis of the implant, through a predrilled alignment cannula on the implant. First, the specimens were fixed in 70% ethanol, dehydrated in graded ethanol (70–99%), embedded in methylmethacrylate, and ground and polished (EXACT-Micro Grinding System) to a thickness of 50 μm. The ground sections were stained with basic fuchsin and counterstained with light green. To ensure that the polyethylene particles were retained in the peri-implant space, polarized microscopy was used.

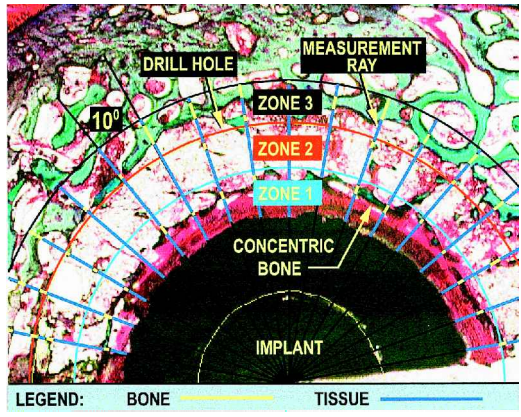


Figure 2. Linear intercept method for determining bone area and contact in defined zones of the drill hole, and for identifying endosteal bone (SEBR) rims.

The procedure was performed blindly and in a random order using one method derived from the linear intercept technique, and another a standard digitizing method. Histomorphometric measurements were divided into 2 zones. Zone (1) was defined as extending from the implant to 50% of the drill gap. Zone (2) was defined as extending from 50% of the gap to 95% of the original drill gap.

Linear intercept method. Rays originating at the center of the implant were tracked every 10 degrees at 2× magnification (Figure 2). Transitions between bone and fibrous tissue along the ray were identified. This method was used to estimate, for each zone, the percentage bone area (percentage of measured bone compared with total length of ray), and presence and location of a rim of sclerotic endosteal bone (SEBR), i.e., a narrow chain of trabeculae (with intermittent breaks) which has formed at a consistent radius from the implant surface.

Digitizing method. This analysis was done on a BioQuant Workstation (R&M Biometrics, Nashville, TN) with a microscope (E400, Nikon Corp., Tokyo) feeding an image to a computer via a video camera (Sony Corp., Tokyo). The analysis quantified the amount of bone in two zones in the gap using the same parameters as the ray method, by tracing features of interest on the video screen. This was also used to quantify the percentage of bone in contact with the implant. BioQuant software translated these tracings into the histomorphometric parameters.

Table 2. Mechanical pushout parameters for stable Ti implants with and without allograft, and for unstable Ti implants without graft, primary and revision, after 4 weeks of implantation. Mean (SEM)

Pushout parameters	Stable		Stable + graft		Unstable	
	Primary	Revision	Primary	Revision	Primary	Revision
Strength (MPa)	0.26 (0.08)	0.16 (0.03)	4.2 (0.93)	1.0 (0.36)	0	0
Stiffness (MPa/mm)	0.91 (0.33)	0.43 (0.12)	19 (3.8)	4.1 (1.3)	0	0
Energy (J/m)	0.81 (0.26)	0.53 (0.10)	13 (2.8)	3.1 (1.0)	0	0

Statistics

ANOVA was employed to evaluate the effects of graft and implant stability under the primary and revision settings. Paired t-tests were used in the within-dog comparisons for the primary setting compared to the revision setting and unpaired t-tests for group comparisons. Statistical significance was assumed for p values < 0.05.

Results

Interfacial shear strength, stiffness and energy (Table 2)

Implant stability was the major determinant of interfacial strength, with unstable implants offering no resistance to pushout (strength, stiffness and energy were zero). If implants were stable, the addition of bone graft significantly increased the shear properties.

Strength of grafted primary Ti implants was more than tenfold greater than ungrafted primary ($p = 0.006$).

Strength of grafted revision Ti implants was sixfold greater than ungrafted revision ($p = 0.05$).

Shear stiffness for grafted primary and revision implants were nine- to tenfold greater than for ungrafted primary ($p < 0.001$) and revision ($p < 0.001$) implants.

Cytokines in joint fluid

The effects of reimplantation (revision), bone graft and implant motion were analyzed separately for the cytokines IL-6β, TNF-α and TGF-β (Figure 3). Separate variance t-tests were done to determine differences between selected groups, with $p < 0.005$ considered as the level of significance.

IL-6 β. If implants were stable-ungrafted, there were no statistically significant differences between

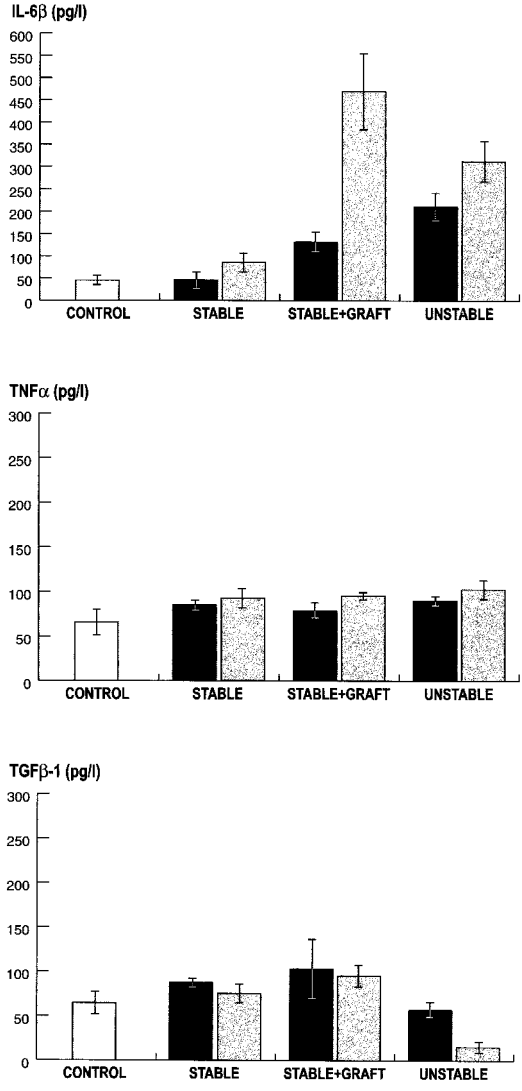


Figure 3. Cytokines IL-6 β, TNF-α and TGF-β in joint fluid from primary (black) and revision (gray) stable implants with and without allograft, and from unstable implants. Control levels are from the unoperated shoulder joint. The separate effects of revision, bone graft, and implant motion are shown.

Table 3. Histomorphometric parameters for stable Ti implants with and without allograft, and for unstable Ti implants without graft, primary and revision, after 4 weeks of implantation. Mean (SEM)

Histomorphometry parameters	Stable		Stable + graft		Unstable	
	Primary	Revision	Primary	Revision	Primary	Revision
Bone ingrowth (%)	0.5 (0.35)	0	31 (4.1)	12 (4.6)	0	0
Bone area in zone 1 (%)	7.7 (1.9)	5.1 (2.5)	33 (6.5)	26 (6.1)	0.46 (0.46)	0
Bone area in zone 2 (%)	20 (4.1)	22 (3.3)	47 (9.8)	33 (2.6)	18 (3.1)	32 (9.4)

the primary setting and control, the revision setting and control, and between primary and revision settings.

In both stable-grafted and unstable implants, however, IL-6β was higher than control and stable-ungrafted implants, respectively. IL-6β was highest in stable-grafted implants, followed by unstable implants, and was lowest in stable-ungrafted. These trends were observed in both primary and revision implants.

TNF-α. There was no statistically significant difference in TNF-α between primary and revised conditions and between primary implants and the control. However, there was a difference between revision implants and control. This was found in stable-ungrafted, stable-grafted and unstable treatment conditions. Moreover, there was no statistically significant difference among these treatment conditions within the primary setting, or within the revision setting, respectively.

TGF-β. If implants were stable-grafted, there was no statistically significant difference between primary and revised conditions and between revised implants and control. However, there was a difference between primary implants and control. Revision unstable implants had lower TGF-β levels than primary unstable implants, and than grafted and ungrafted revision stable implants. Primary unstable implants had lower TGF-β levels than primary stable implants. The sole treatment condition where TGF-β levels were elevated, was revision grafted implants which were higher than in the control.

Histomorphometry (Table 3)

Surface in contact with implant. The addition of bone graft to stable Ti implants increased the bone area in contact with the implant, in both the revision and primary settings ($p = 0.005$). There was a consistently higher percentage of bone in contact in primary implants than in revision ones, for all treatment groups. There was no bone in contact with any unstable implants, whether primary or revised.

Area in zone 1. There was no bone area in zone 1 in unstable implants, whether primary or revised. Bone area in zone 1 in grafted stable primary implants was fourfold greater than in ungrafted stable primary implants ($p = 0.02$). Bone area in zone 1 in grafted stable revision implants was fivefold greater than in ungrafted stable revision implants ($p = 0.01$). There was no significant difference in bone area in zone 1 between the primary and revision settings.

Area in zone 2. There was no statistically significant difference in bone area in zone 2 between ungrafted stable implants and unstable implants, whether primary or revised. There was a twofold greater bone area in zone 2 (not significant; $p = 0.06$) in grafted stable primary implants compared to unstable primary implants, and no significant difference in zone 2 in unstable revision implants. There was no significant difference between primary and revision conditions in any of the treatment groups.

Presence of sclerotic endosteal bone rims (SEBR). Qualitatively, there were more SEBR rims seen in revision implants than in primary implants. Their location was mainly in zone 2 and at the drill hole zone. An additional SEBR at the drill hole, as quantified by the percentage of rays with bone in the drill region, was greater in revised (Ti: 51% of the rays with bone) than in primary (Ti: 24%). No SEBR were identified in the primary grafted implants and only 28% of the revised grafted implants had SEBR in the gap.

Discussion

Revision implants were less robust than primary implants. Allografting was positive in both primary and revision implants. Unstable implants reduced the quality of implant fixation, with a more pronounced detrimental effect in the revision setting than in the primary one. The most favorable combination of conditions was a grafted primary implant under stable conditions. The most unfavorable combination was a revision implant under unstable conditions.

We found that the major differences between primary and revision implants were that revision implants had lower mechanical pushout parameters, less bone area in contact with the implant, more SEBR rims (particularly in the drill hole), and higher levels of inflammatory cytokines and lower levels of anabolic cytokines. Unstable implants lowered the level of the anabolic cytokine TGFβ, and increased the levels of the pro-inflammatory cytokines IL-6β and TNF-α. Such combined effects would be expected to reduce the ability of the bone further in the revision setting to stabilize the implant by producing bone at the implant surface.

These findings for revision and unstable implants, of reduced mechanical integrity of the interface, inferior bone distribution in the gap, and the inflammatory and anabolic cytokine profiles, are in agreement with clinical studies showing inferior interface integrity and reduced longevity for revision implants. They also further support the use of our revision protocol as a relevant model to evaluate treatment methods of improving the integrity of the implant-bone interface.

Our study showed consistently better fixation with grafting, which may be partially due to its ability to produce a favorable distribution of bone in the gap by providing a satisfactory surface for bone growth. Bone in close proximity to the implant (in contact, or in zone 1), and the lack of a sclerotic endosteal bone rim (SEBR) were found to be positive bone distribution patterns and were common in grafted implants. Furthermore, the detrimental presence of SEBR was reduced with grafting. At the same time, pushout strength was increased with grafting, in both the primary and revision settings. Although grafting increased the pro-inflammatory IL-6β levels, it also restrained the potentially detrimental reduction in TGF-β associated with revision and instability.

Bone grafting is widely used clinically to improve the success of revision arthroplasty and restore bone stock. Our results agree with the satisfactory clinical results of allo- and autologous bone grafting in primary implants, which have ranged from 85% to 100% with 6- to 72-month follow-up (Gross et al. 1985, Head et al. 1987, Oakeshott et al. 1987, Søballe et al. 1992b). Autograft has been found better in terms of antigenicity, healing, and regeneration capacity (Goldberg and Stevenson 1987, Stevenson et al. 1992, Turner et al. 1993). It has also proved better than freeze-dried allograft (Knienapfel et al. 1992), and the combination of biphasic ceramic (composed of hydroxyapatite and tricalcium phosphate) and autogenous graft has been found better than autograft alone (Kang et al. 1991), but not around porous-coated implants. Hydroxyapatite improved the ability of allograft to incorporate, but by itself was equally successful in producing anchorage (Søballe et al. 1992b). Recent clinical studies suggest that the appearance of specific anti-HLA antibodies and cell-mediated immune reactions caused by allogenic bone grafts is not clinical evidence of an adverse effect of the graft. In our study, although inflammatory cytokines were increased with allograft, a moderate positive effect on an anabolic cytokine was also noted with allograft. Bone graft can be expected to enhance implant fixation via its role in providing surfaces for differentiated mesenchymal cells, and also for providing bone morphogenetic proteins and other growth factors to aid in differentiating osteoclasts.

In summary, using the revision protocol in Part I, we have shown its applicability to titanium plasma-sprayed primary and revision implants. We documented the use of allograft in improving fixation of revision implants, suggesting that allograft may be particularly important in improving the success of revision implants. We also showed the detrimental effects of implant motion. While it is not unexpected that implant motion is undesirable, this suggests the importance of establishing a stable interface at the time of surgery, to produce a favorable and robust environment for high-quality implant fixation (in both the primary and revision settings).

The results of this study must be interpreted with its limitations in mind. Although this experi-

mental implant has been used successfully for over a decade to study the effects of mechanical interface conditions and implant surface characteristics, it represents the tissue response under its particular configurations (0.75 mm gap, loaded, with or without 500 μm motion and with or without allograft, in the cancellous bone of the distal femoral condyle of the dog). Although it is smaller than a typical joint replacement implant, it can realistically represent the mechanical conditions at portions of a clinical implant's interface with the bone, as well as the loading conditions that an implant can undergo. In addition to being a loaded implant, a major advantage of this device is its ability to control the mechanical conditions, and isolate their contribution to the periprosthetic tissue response. In particular, it allows comparison of the primary and revision setting, since they have identical geometries and loading conditions. This facilitates the study and ranking of the effectiveness of various treatment strategies to improve the outcome of revision implants.

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