Histological and biomechanical analysis of bone and interface reactions around hydroxyapatite-coated intramedullary implants of different stiffness: a pilot study on the goat


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Histological and biomechanical analysis of bone and interface reactions around hydroxyapatite-coated intramedullary implants of different stiffness: a pilot study on the goat

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We hypothesized that reduced stem stiffness of orthopaedic implants contributes to a high risk of loosening, since interface stresses and relative motions may exceed a tolerable range. To study this hypothesis, three types of load-bearing implant with different stiffness were inserted into the tibia of the goat. Histological analysis was performed of bone repair after insertion of the implant, bone ingrowth, interface disruption and loosening. A finite element model of the configuration provided the quantitative range of interface stresses and relative motions for the present experiment. The implants were made out of stainless steel, hollow titanium and a thin titanium core covered with a polyacetal coating. The stiffness ratios of these implants were approximately 10:4:1, respectively. All implants were coated with a layer of hydroxyapatite (HA) in order to minimize the possible biological effects of the different implant materials. Irrespective of the type of implant, there was a repair phase that lasted 6-12 weeks. The stiff implants functioned well. Large areas of bone bonding to the HA layer were found after the repair phase at 12 weeks postoperatively. After 24 weeks, some signs of loosening were observed. More loosening occurred with the hollow titanium and polyacetal implants, mainly during the repair phase. Three hollow titanium and three polyacetal coated implants survived this period, and were killed after 24 weeks. The integrity of the HA layer at the bone-implant interface of the titanium implants was good. In the polyacetal implants, the repair reaction of the cortical bone was incomplete. Bone ingrowth into HA was largely lacking. In conclusion, we found significant differences in the repair and interface reactions around implants of different stiffness. Stiff implants showed favourable initial interface conditions for bone ingrowth. Intermediate and flexible implants provoked unfavourable interface conditions for initial bone ingrowth. The finite element study showed that the flexible stems produce larger micromotions and higher interface stresses at the bone-prosthesis interface than the stiff stems, indicating an explanation for the histological findings. © 1997 Elsevier Science Limited. All rights reserved

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Irrespective of the type of hip prosthesis, cemented or non-cemented, the main reason for revision is aseptic loosening of one of the components. Different prosthetic designs have different failure rates. The failure scenario of prosthetic component loosening is complex, since interacting causative biological and mechanical factors are involved. Excessive stresses at the bone-prosthesis interface in cemented and non-cemented prostheses may play a very important role in the failure mechanism.

Adaptive bone remodelling induced by unnatural stresses in the bone may be detrimental for the survival of prosthetic components. To avoid proximal bone atrophy induced by stress shielding, femoral stems were developed that have a relatively high flexibility, matching that of bone (isoelectic prostheses). Indeed, these isoelectic stems showed relatively little proximal calcar resorption, but the failure rates of these types of
prosthesis are relatively high compared to similarly designed high-modulus stems\textsuperscript{12-14}. One of the prerequisites for long-term success of prosthetic implants is a stable bone–implant interface\textsuperscript{12-16}. Relative motions that surpass 28-40\(\mu\)m will prevent bone ingrowth in porous coated prostheses\textsuperscript{17,18}.

It was hypothesized that a stiff stem, provoking relatively small micromotions, has favourable mechanical conditions at the interface for successful bone ingrowth. However, they increase the risk for long-term proximal bone resorption due to stress shielding of the bone. Alternatively, flexible stems will reduce stress shielding, but will generate larger micromotions at the bone–prosthesis interface, and if implanted in a press-fit manner will generate higher interface stresses with the risk of failed bone ingrowth\textsuperscript{19-21}. Theoretically, an intermediate prosthetic stiffness could balance stress shielding, interface motion before ingrowth and interface stresses after ingrowth\textsuperscript{19}.

The events at the bone–prosthesis interface of stems with different stiffness before, during and after the ingrowth process are still poorly understood. The present study was performed in order to describe and understand the processes at the bone–prosthesis interface in detail and to enlarge our knowledge about the failure mechanisms of prosthetic components. More specifically, it was investigated what the tolerable range of implant stiffness is in order to balance interface relative motion and stress.

Three types of implant with variable stiffness were inserted into the tibia of the goat. The bonding characteristics were analysed histologically at various postoperative periods. Interface stresses and micromotions for the three types of implant were estimated using three-dimensional finite element analysis.

**MATERIALS AND METHODS**

Eighteen solid stainless steel, 10 hollow titanium and 10 flexible polyacetal implants with a titanium core were placed in the right tibia of the skeletally adult Dutch milk goat (Capra Hircus Sana). All goats weighed between 45 and 55 kg (51.3 ± 4.3 kg) and were operated upon using standard general anaesthesia and disinfection techniques. After a segmental resection of about 15 mm of the mid-shaft part of the tibia, the distal medullary canal was drilled and reamed with conical intramedullary reamers and cleaned. The implant consisted of two parts: the tapered-shaped distal part was 55 mm long, with a proximal diameter ranging from 8.7 to 11.2 mm and a distal diameter 2 mm smaller. All implants (stainless steel, hollow titanium or polyacetal with a titanium core) were smooth and had a similar surface topography. All implants were fully coated with hydroxyapatite (HA) with a crystallinity of 70\% (CAM implant service BV, Leiden, The Netherlands). The stiffness ratios in bending were 10:4:1, respectively. Before insertion of the implant, the medullary cavity was prepared with tapered rongeurs, ensuring adequate primary press-fit conditions of the implant (as tested manually). After insertion of the implant, rotational stability was ensured by a proximal locking nail. The proximal part of the implant was fixed with bone cement into the proximal tibia and connected to the distal part (Figure 1). Immediately postoperatively, all the goats were radiographed in the antero-posterior and lateral directions. They were first kept in a hammock for 2 days and then allowed full weight-bearing. Postoperative clinical performance and weight-bearing were judged by a rating system\textsuperscript{22} using a scale from 0 (not used at all) to 4 (normal walking and standing). To allow for qualitative evaluations of bone ingrowth and remodelling, all the animals were labelled with three types of fluorochrome\textsuperscript{23-25}, namely oxytetracycline (20 mg per kg per day, for 7 days after the operation), Xylenol Orange (20 mg per kg per day, for 4 days in the middle of the postoperative period) and Calcein Green (20 mg per kg per day, for 4 days directly before killing).

Eighteen stainless steel implants were placed, intended for analysis after 3 (three implants), 6 (four implants), 12 (three implants), 24 (four implants) or 48 (four implants) weeks after implantation. Due to loosening, this investigation scheme could not be fully maintained. Ten hollow titanium and 10 polyacetal implants were intended for evaluation after 24 and 48 weeks (five for each time period), but since most fixations failed prematurely in these series, only three could be analysed after 24 weeks for both types of implant. The animals were heparinized and killed by an overdose of sodium pentobarbitone. The hindlegs were perfused via the descending aorta with physiological saline until all blood was removed. The perfusion

![Figure 1](image-url)  
**Figure 1** Experimental configuration placed in the tibia of the goat.
was continued with 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). Tibias were harvested by careful exarticulation, with an ample musculature cover, leaving the periosteum intact. Fixation was continued for at least 10 days by immersion in the same fixative.

After fixation, tibias were X-rayed and sectioned transversally by means of a water-cooled saw in 3-mm-thick slices. The slices were X-rayed again. These slices allowed observations along the entire length of the implant. Three levels were investigated in detail, namely proximally, mid-shaft and distally, i.e. immediately proximal to the tip of the implant. To assess the integrity of the HA layer covering the implant and to evaluate bone ingrowth into the layer of HA, one surface of each slice was polished and studied with incident blue and green light, with or without previous staining. For histology, the slices were decalcified in 25% ethylene diaminetetra-acetate under radiographic control, embedded in poly(methyl methacrylate) (PMMA), thin sectioned (7 μm; Jung 2050 microtome) and stained with haematoxylin and eosin (HE). For fluorescence microscopy, 3-mm-thick slices were dehydrated without any pre-embedding staining, embedded in PMMA and sectioned (30 μm) with a rotating water-cooled diamond saw.

A finite element computer model of the bone implant configuration was constructed. Computer tomography (CT) scans of a representative tibia were made at serial slices with 2-mm intervals and a finite element (FE) model constructed from the three-dimensional CT data such that the geometry of the FE model represented the scanned tibia accurately. The mechanical properties of the tibial cortex were obtained from experimental mechanical tests. This provided elastic moduli in the longitudinal \( E_l \), radial \( E_r \) and circumferential \( E_c \) directions \( E_l = 23.2 \text{ GPa}, \ E_r = 5.3 \text{ GPa} \) and \( E_c = 10.8 \text{ GPa} \). The Poisson ratio was given a value of 0.3. The external loads acting on the tibia were determined from strain gauge measurements on the tibia of a goat during \emph{in vivo} experiments whereby several functional gait patterns were recorded.

A representative loading case from these data was taken and applied to the computer model (Figure 2). The stiffness of the stem was taken in accordance with the stiff stainless steel implant and the flexible polyacetal implant with the titanium core, the two extreme cases in the experiment. The interface conditions were assumed to be either fully bonded or debonded with a frictionless interface, again the two extreme situations possible. In this way the range of interface stresses and relative micromotions for the experimentally used implants could be estimated.

**RESULTS**

**General and clinical observations**

All the goats were ambulated and allowed full weight-bearing after 2 days in a hammock. The clinical score was 2.9 (± 0.4) after 1 week, 3.75 (± 0.4) after 2 weeks and 3.9 (± 0.3) after 3 weeks; this indicates that most goats achieved normal weight-bearing patterns with normal ranges of motion. No clear differences were found between the different types of implant during this period.

**Stainless steel implants**

Two of the 18 implants failed shortly after implantation, due to fractures of the connection between the proximal and distal parts of the implant (one in the 12-week group and one in the 48-week group). They were not analysed further. Four other implants showed loosening of the cemented proximal part but could be further analysed, since the distal implant remained well fixed. One case of gross implant loosening occurred after 8 weeks in the 24-week group and two in the 48-week group after 5 and 6 months, respectively. This means that three (out of three) cases at 3 weeks, four (out of four) at 6 weeks, two (out of three) cases at 12 weeks, three (out of four) at 24 weeks and one (out of four) cases at 48 weeks could be analysed.

**Hollow titanium implants**

All except three of the 10 implants failed by gross loosening of the distal stem. One failed after 2 weeks and six between 8 and 12 weeks (8.5 ± 3.43 weeks). The remaining three were analysed after 24 weeks.
bone in direct contact with the HA partly filled by new bone. Local osteoclastic bone lysis labelling and routine histology showed that the necrotic bone, directly facing the HA layer, and creeping substitution of the necrotic endosteal cortical bone had become completely revascularized. The process of creeping substitution was completed and the implant was surrounded by a layer of newly formed bone. The HA layer was largely intact (Figure 4K, L). Almost complete osseous integration was found between the HA and the bone. Foci of bone remodelling at the interface, labelled with Calcein Green, had locally impaired the integrity of the HA layer (Figure 5A, B). In these locations the HA layer had disappeared, probably as an effect of the acid environment produced by the osteoclasts in the process of bone resorption29.

The first signs of loosening of the HA layer were observed in all three specimens in the 24-week group, particularly around the distal half of the implant. Between the HA and the metal, an acellular layer of basic fuchsin-stained fibrin-like material was present, or a thin soft tissue interface had formed (Figure 5C, D). Locally, the HA had disintegrated and had been phagocytosed by macrophages (Figure 5D). Metal abrasion particles were seen in the interface as well (Figure 5E). Since this type of implant has a non-articulating connection, it seems likely that the particles were formed by fretting of the distal part of the implant to the cortical bone.

In the one specimen that was killed after 48 weeks, no radiographic nor functional signs of loosening were detected, but histology showed a pronounced process of interface loosening. Proximally, the cortical bone had become very thin, probably by stress protection of the relatively stiff implant (Figures 4A and 5E). Distally, the soft tissue interface was thicker between the implant and the bone. HA particles had become engulfed by macrophages. Many dark metal (abrasive) particles were also present (Figure 5F).

Hollow titanium implants
The histology of the failed implants was characterized by increased bone turnover at the implant-bone interface, bone lysis and soft tissue interface formation (Figure 6B, C). Only local remnants of the HA coating were found. Goats with well-functioning implants were killed and examined after 24 weeks. The interfaces of these implants were characterized by an intact HA layer, without delamination or soft tissue interface formation (Figure 6D, E). Signs of bone remodelling with involvement of the HA layer were scarce. No proximal cortical resorption, as a possible effect of stress shielding, was observed.

Polyacetal implants
The histology of the interfaces of the three implants that had functioned well for 26 weeks showed characteristic patterns. In the proximal sections, Calcein Green labelling and routine histology showed that the creeping substitution of the necrotic endosteal cortical bone had stopped at a distance from the bone-implant interface, leaving a layer of circumferential necrotic
Figure 4  
A. X-ray 27 weeks postoperatively of stainless steel prosthesis in the tibia of the goat. The lower part is the conical shaped HA-coated experimental prosthesis inserted into the conical reamed medullary canal of the tibia; the upper part is cemented to the proximal tibia. Original magnification ×1.1.  
B. X-ray of thick sections at proximal (A), mid-shaft (B) and distal levels (C) along the prosthesis of the 8-week group. Level D is distal to the tip of the prosthesis. Original magnification ×1.1.  
C. Periosteal reaction 6 weeks after the operation labelled with oxytetracycline. Original magnification ×50.  
D. Transition between living and necrotic bone, 6 weeks postoperatively. Note the osteoclastic bone resorption (arrow) and empty osteocyte lacunae. Original magnification ×160.  
E. Low-magnification photograph (original magnification ×10) of an unstained section of tibia 1 week after insertion of the prosthesis, showing the difference in contrast between dark necrotic bone (NB) around the prosthesis and lighter living bone (LB) in the peripheral cortex. The prosthesis is not visible, due to the low level of reflected light.  
F. Basic fuchsin-stained thin section (20 μm) of bone remodelling 6 weeks postoperatively. There are many remodelling cavities (arrows). Original magnification ×60.  
G. X-ray of 100-μm-thick section 6 weeks postoperatively, after removal of the prosthetic material. Note the creeping substitution of the cortical bone. Original magnification ×4.  
H. Bone–prosthesis interface after 6 weeks, visualized with incident light. Note that the gap between the bone and HA layer is filled by viable bone. Original magnification ×120.  
J. Approximately the same location as H, but 20-μm-thick section stained with basic fuchsin. Note the complete osseous integration of the cortical bone with the HA layer. Original magnification ×80.  
K. Interface between the bone and HA layer 12 weeks after implantation. Note the osseous integration of the HA layer with new bone. Original magnification ×50.  
L. Enlargement of the encircled area in K. Original magnification ×125.
bone. Osteoblastic and osteoclastic cellular dynamics were lacking and the peri-implant osteocyte lacunae were empty. This resulted in a still considerable necrotic zone around the implant after 24 weeks (Figure 7C, D, F). Locally, fuchsin-stained microfractures were seen, most of them perpendicular to the surface of the endosteal bone (Figure 7E, F). The integrity of the HA layer was lost. Instead, a layer of acellular fibrin-like material was present, with many HA particles included (Figure 7C, D). The proximal bone cortex had thickened compared to the preoperative situation (Figure 7A). At mid-implant level, more active bone remodelling cavities were found and locally new bone had reached the bone–implant interface. At these locations active bone lysis and interface formation were observed, indicating that the implant was partly surrounded by a fibrous tissue interface and partly by necrotic bone. No microfractures were found at mid-shaft levels. At distal levels around the implant the creeping substitution...
Implant stiffness-dependent bone and interface reactions. P. Buma et al.

Figure 7 A, Polyacetal HA-coated prosthesis with titanium core. Note the proximal cortical thickening (arrow). Original magnification x1.1. B, Cross-sections of the same specimen as A. Original magnification x1.1. C, D, The interface 24 weeks postoperatively between the prosthesis and bone in non-decalcified (fuchsin stained; D) and decalcified (HE stained; C) sections. Note the many loose HA crystals (arrows) embedded in a fibrin-like material. Original magnification x150. E, Microfracture in endosteal bone (arrowheads). Original magnification x80. F, Basic fuchsin-stained proximal thick undecalcified section showing small microfractures (arrowheads). G, Same section, but with incident light for fluorescence detection, showing scarce Calcein Green-labelled active remodelling cavities at a distance from the implant-bone interface. Original magnification x80. H, Fuchsin-stained section showing soft tissue interface at bone-implant interface in distal section. J, Same section with fluorescence of Calcein Green labelling. Note active bone remodelling at bone–prosthesis interface. Original magnification x80.

had reached the bone–implant interface circumferentially. No bone ingrowth into the disintegrated layer of HA was found, but the implant was surrounded by a fibrous tissue interface (Figure 7H, J).

Finite element model analysis

The FE analysis showed a proximal and distal interface stress peak for the fully bonded stem, which is a typical pattern for such an implant configuration. For the flexible implant the proximal stresses were the highest and for the stiff implant the distal stresses were higher (Table 1). Overall, the flexible stem developed higher stresses. For the unbonded analysis the relative motions between stem and bone were evaluated. The flexible stem provoked 4.3 mm motion at the proximal region versus 3.8 mm for the stiff stem.
DISCUSSION

If one wishes to investigate implant-loosening phenomena, then the experimental model must indeed allow for loosening to occur. In that respect, our model proved quite adequate. Apparently, the forces in the tibia are high in relation to the fixation capacity of the stem, as was also shown in an earlier analysis of implants with a similar model. Hence, the animal model that was developed creates worst-case conditions which are well suited to test implant-loosening scenarios. Moreover, the implant is fixed in cortical bone with a less effective interface revascularization potential compared to trabecular bone, which makes the conditions for ingrowth even worse. On the other hand, however, these differences must be kept in mind when translating the results on osseous integration patterns to the human clinical conditions of a hip replacement, where revascularization occurs rapidly and trabecular fixation is important.

The purpose of this study was to assess the effects of stem stiffness on cortical repair reactions, bone ingrowth, interface disruption and the stem loosening mechanism. The mechanical FE analyses had shown that the flexible stems produced higher interface motions if unbonded and higher interface stresses if bonded. The specific findings in this study confirmed earlier findings of other stem configurations. As a consequence, one would expect more cases of failed ingrowth and/or late loosening around flexible, as compared to stiff, stems. This was indeed confirmed by the experiments. Although all stems were HA coated in order to standardize the mechanical and biological characteristics of the surface, the bonding strength of this coating to the implant was probably not equal for stainless steel, titanium and polyacetal. It is speculated that, particularly in the latter case, the bonding is weak. Also, due to the flexibility of the polyacetal, disintegration of the HA layer by mechanical loading, deformation and abrasion of HA particles may have played a role in the process of loosening as compared to the other stem types. Despite the differences with respect to coating fixation, the comparisons between all three types of implants tested in this study point in the same direction, where the general effects of stem stiffness are concerned.

All implants were coated with HA. The effects of HA on new bone formation are well documented. HA coatings accelerate the ingrowth process and thus increase the direct postoperative interface strength of the prosthesis with the bone. In the present series, this positive effect of HA could not take place directly after the operation, because viable bone could only reach the HA-bone interface after creeping substitution of the necrotic cortical endosteal bone was completed. Necrosis could have been the result of the heat produced by the drilling procedure, but since the drills were cooled, it seems more plausible that avascularity due to the trauma is the main factor involved in the generation of the endosteal necrosis. For the stainless steel (rigid) stems, the period that the endosteal bone surface stays fully necrotic appears to be approximately 6 weeks. The stem fixation seems to be secure during this period, but no ingrowth or bonding can take place. The creeping substitution starts from the vascularized periosteal side and slowly moves in the direction of the stem. Generally, this process of creeping substitution progressed at a rate of approximately 120 μm per week in the direction of the implant in the case of the stainless steel stems. The first apposition of new bone against the layer of HA was observed after 6 weeks, but due to the varying degree of endosteal necrosis this period also varied and could take as long as 12 weeks. Consequently, the prostheses were only partly anchored to the living bone in this period. For the ingrowth process of the bone to the implant, and hence for the process of osseous integration, this is a very vulnerable period, since the creeping substitution creates quite a porous bone interface and the mechanical press-fitted fixation is endangered. During this ingrowth period, the well-known positive effects of HA on implant-bone bonding could be confirmed. Small gaps (if present) were rapidly filled with new bone, resulting in almost 100% osseous integration of the bone with the HA layer 12 weeks after the operation. If this period is survived, another potential loosening mechanism follows for the rigid stem. After 24 weeks, a characteristic failure pattern was found: delamination of the HA-metal bond with fibrous interface formation at the distal implant. This was precisely the location where the highest interface stresses were found in the FE analysis with the bonded interface. It seems likely that the combination of high tension and shear stress (Table 1) provokes debonding of the interface. Once loosening starts, micromotions can occur which can further generate loosening and soft tissue interposition. This fits with the observed osteoclast-mediated dissolution of the HA layer in the remodelling cavities at the HA-bone interface. This led to degradation of the HA layer and reduced the percentage of bonding between the bone and the HA. This process was also observed in human retrieval specimens of HA-coated prostheses. Then, if the HA layer is not replaced by a bony bond, interface stresses in the remaining bonded HA will increase and delamination of the HA at the implant–HA interface will inevitably occur if a critical level of interface stress is surpassed. Of course, fatigue failure of the HA layer by repetitive loading can also contribute to the loosening process.

<table>
<thead>
<tr>
<th>Young's modulus (GPa)</th>
<th>Proximal compressive</th>
<th>Proximal tensile</th>
<th>Proximal shear</th>
<th>Distal compressive</th>
<th>Distal tensile</th>
<th>Distal shear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexible stem</td>
<td>21</td>
<td>11.7</td>
<td>12.9</td>
<td>32.9</td>
<td>1.6</td>
<td>3.4</td>
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<tr>
<td>Stiff stem</td>
<td>210</td>
<td>3.2</td>
<td>3.4</td>
<td>7.7</td>
<td>5.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Table 1 Peak interface stresses around the flexible and stiff stems as calculated in the finite element analysis.
After delamination the implant is again anchored to the bone solely by the press-fit mechanism. Further micromotions during mechanical loading of the implant will induce further soft tissue formation and possibly HA degradation. The end result of this process will be a loose stem in a soft tissue interface. A vicious circle of bone destruction, larger micromotions, subsidence, particle formation and further prosthetic loosening will then be activated, with its well-known characteristics. In this phase of loosening, fretting of the prosthesis to the cortical bone can induce a metal wear reaction, as observed in the specimens that had been in situ for 24 and 48 weeks. Similar phenomena were observed in a retrieved failed HA-coated hip prosthesis from a patient.

The flexible and intermediate implants provoked more cases of early loosening as compared to the stiff implants. In the mechanical analyses it was found that the flexible stems generate higher micromotions in the unbounded case and higher interface stresses in the bonded case. Kuiper and Huiskes reported similar findings for a model of a human femoral stem which included friction and various dynamic loading cases. The relative motions at the interface calculated in the present model seem unrealistically high. The presence of friction between stem and implant will certainly reduce this drastically. From the findings of Kuiper and Huiskes it was concluded that, in the case with friction included in the analysis, the relative difference in interface motion between a rigid stem and a flexible stem was quite large, up to three-fold at the proximal region. It seems questionable whether the flexible implants grow in at all in the present study. The histological findings from three specimens with the flexible polyacetal implants indicate that there is a different creeping substitution and ingrowth pattern for the flexible implants. Fewer active remodelling cavities were found, indicating that the creeping substitution of the necrotic endosteal bone took place irregularly and at a much lower rate. The three flexible implants which survived a period of 24 weeks were still surrounded by large areas of necrotic bone. At the few locations where new bone had reached the fragmented HA layer, no bone ingrowth had occurred. Instead, bone erosions were found at these locations. The reason for this altered pattern of creeping substitution is unclear, but based on the present findings we hypothesize that the stresses in the bone play an important role in the development of the creeping substitution process.

CONCLUSIONS

The present results give evidence that the stiffness of the prosthetic material is a very important factor for the success of an intramedullary stem. Rapid osseous integration of the prosthesis with the bone was achieved with a stiff stem. Intermediate stiffness was found to be a favourable condition for long-term lasting bonding between the HA layer on the stem and the bone, but there was a considerable risk of failure during the ingrowth period, in particular during the period that creeping substitution had reached the implant surface. In the stainless steel group the creeping substitutions generally progressed at a speed of 120 μm per week. In the flexible stem group the creeping substitution progressed much slower. The bone stresses and the relative displacements at the interface between the flexible stems and the bone were probably too large for proper bone ingrowth into the layer of HA, and as a consequence most flexible implants failed.

REFERENCES


