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Migration and inducible displacement of the Miller-Galante II and Freeman-Samuelson hydroxyapatite knee prostheses

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Introduction: A minimum of early migration and inducible displacement are good prognostic factors for future implant outcome. The purpose of this study was to compare migration and inducible displacement for two different tibial component surfaces. The Freeman-Samuelson hydroxyapatite coated (F/S-HA) (Protek, Bern) prosthesis and the Miller-Galante II (MG II) (Zimmer, Warsaw) have both a central stem, pegs and four screws for initial stability. The F/S-HA prostheses is coated with hydroxyapatite while the MG II rely on a titanium fiber mesh surface for bone ingrowth.

Patients and methods: 51 knees with osteoarthritis were randomly allocated to one of the prosthetic systems. Within the first week and at 2-, 6- and 12 months post operative radiographs for RSA (roentgenstereophotogrammetric analysis) were taken. In addition at 2 and 12 months each knee was examined with RSA exposures in the standing weight bearing position with internal and external rotation torque of 10 Nm applied to the foot. Inducible displacement of the tibial component was calculated. The accuracy of the method is 0.1° for rotation and 0.2 mm for translation. Maximal total point motion (MTPM) was used to denote magnitude to motion of the marker which moved the most irrespective of direction.

Results: Clinically there was no statistical difference between the two groups. The mean MTPM at 2, 6 and 12 months was 0.8 mm (SD 0.58, 0.38, 0.59) for the MG-II knees and the corresponding values for the F/S-HA-knees were 0.5, 0.6 and 0.7 (SD 0.35, 0.38, 0.28). The MG-II prostheses displayed more subsidence and anterior lift than the F/S-HA component ($p \leq 0.01$ Student t-test). At two months the F/S-HA implants displaced smaller translation at internal rotation torque ($p \leq 0.05$) and smaller rotation about the sagittal axes at external rotation torque ($p \leq 0.02$). At one year the FS-HA prostheses displayed smaller rotation about the transverse axes at internal rotation torque ($p \leq 0.02$). The

MG-II group had less inducible displacement at one year compared to the two months follow-up, in the F/S-HA-group there was no change.

Discussion: Both implant systems displayed small inducible displacement and small migration rates. These values are smaller than previously reported for most designs. The F/S-HA prostheses had less migration and inducible displacement at 2 months which may indicate a faster induction of bone apposition. The more constrained articular surface of the tibial component for the FS-HA knee did not affect the interface stability. The stable fixation of these two prosthetic systems points towards bony apposition and may predict good long term results.

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Accuracy of migration analysis in total hip arthroplasty—digitised and conventional radiography vs. radiostereometric analysis

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We assessed the accuracy of migration measurements on conventional and digitized radiographs of total hip arthroplasties by comparing the results with radio-stereometry, (RSA). 4 stem and 3 acetabular designs were studied. 2 of the stem designs and 1 cup design were uncemented. 180 manual and 202 digitized measurements were done on 120 conventional radiographic examinations.

The readings on digitized radiographs did not differ from the manual measurements on the same radiographs. A comparison of the measurements from plain radiographs and with RSA of the femoral stems revealed an accuracy, (absolute mean + 2 SD) of between 3.9 and 12.3 mm depending on the choice of landmarks. The greatest accuracy was obtained by using tantalum markers inserted into the greater or lesser trochanter and the shoulder of the stem. The most me-

dial point of the lesser trochanter proved to be the best bony landmark. Measurements, including the centre of the femoral head and the greater trochanter, were associated with poor accuracy. The accuracy for horizontal cup migration varied from 4.4 to 6.5 mm and the accuracy for vertical migration varied between 4.4 and 6.3 mm.

The intraobserver error (2 SD) ranged from 1.6 to 5.6 mm. The corresponding figures for the interobserver error were 2.6 and 6.6, respectively. One of the cemented cup designs was associated with inferior accuracy. Stem design did not affect the accuracy.

Early failure of PMMA precoated femoral components in total hip arthroplasty

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Introduction: Prostheses with PMMA precoating have been developed to further improve the fixation between cement and prosthesis. This paper reports 9 cases with early failure of PMMA precoated femoral components within three years after primary total hip arthroplasty (THA).

Methods: Nine patients (68 (63–71) years) with osteoarthritis underwent primary THA using a PMMA precoated stem. In all cases, a noncemented acetabular component (Harris-Galante) and cemented PMMA precoated Harris-Galante femoral component with a 28 mm head (Zimalloy[®]) were used. The femoral component was cemented with vacuum mixed Simplex[®] using distal plugging and proximal pressurization. Initially the patients did well, but 1–2 years after surgery they complained of hip pain. Radiographs showed progressive osteolysis around the femoral component. Sedimentation rate and C-reactive protein was normal in all cases. **Revision THA** was performed 37 (15–48) months after primary arthroplasty. The femoral components were debonded from the surrounding cement mantles, were surrounded by a thick fibrous membrane, and were easily removed. Cultures from the joint, femoral canal, and prosthesis surface were all negative. Revision THA was uneventful and all patients are now doing well, 10–24 months after revision.

Results: *Radiologic examination:* after primary THA all stems were well cemented with no radiolucent zones. Before revision, all stems were surrounded by extensive progressive osteolysis and all stems had migrated. Debonding between prosthesis and cement was obvious in most cases. *Histological findings* (from fibrous membrane and joint capsule) were systematically evaluated using conventional and polarized light microscopy. The membrane consisted of fibrous tissue with many histiocytes and foreign body giant cells. Brown barium containing particulate PMMA was seen in all membranes. There were no signs of acute inflammation, and no polyethylene (PE) nor metal particles at the microscopic lev-

el. There was no Willert phenomenon which would have suggested submicron PE particles.

Discussion: This early osteolysis does not seem to be primarily caused by PE wear debris, as PE particles were not seen in the membrane, and the liner was not markedly worn. An interesting finding was debonding at the cement-prosthesis interface, which might be the primary feature in the initiation of these prosthetic loosening, and may lead to additional mechanical failure of the cement mantle. These changes might explain the abundant presence of particulate PMMA in the fibrous membrane surrounding the cement mantle.

Changes in bone mineral densities after two different uncemented THA's— preliminary results in a randomized trial

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Introduction: The preliminary results of adaptive bone remodelling after THA in a prospective randomized trial with two different designs of femoral components are presented.

Material and methods: Eleven patients were randomized to either Bimetric primary femoral stem (n=6) or Ranavat stem (n=5). The stems were collarless with proximal porous coating and implanted using uncemented technique. Bone mineral density (BMD) were measured using DEXA, at calcar and the minor and greater trochanteric regions. The measurements were performed postoperatively and with follow-up after 3 and 6 months.

Results: In the calcar region the BMD had decreased 13.0% 6 months after implantation of the Ranavat stem and 15.9% after implantation of the Bimetric stem. At the lesser trochanter a decrease of 18.1% after Ranavat and 2.6% after Bimetric was seen. At the greater trochanter the decrease was 10.1% after Ranavat and 9.3% after Bimetric. In the proximal tibia of the operated leg a decrease of 7.4% was seen after Ranavat and an increase of 3.0% after Bimetric. In the contralateral healthy leg a decrease of 8.0% was seen after Ranavat and 2.7% after Bimetric.

Conclusion: These preliminary results may indicate that the design of the Ranavat stem causes more stress shielding at the lesser trochanter compared to the Bimetric stem. However these results are preliminary and more patients and longer follow-up are needed before any definite conclusion can be made.

Inter/intra-observer variation in the evaluation of radiographic healing after posterolateral lumbar fusion (PLF)

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Introduction: During recent years PLF with or without pedicle screws has become more frequently used in the treatment of segmental instability combined with central or lateral nerve compression of the lumbar spine. Published radiological success rates for PLF varies from 60% to 95%. However, the question remains whether the x-ray assessment is reliable due to implant overlapping the bone graft or presence of pseudarthrosis in oblique planes. Fusion criteria are often poorly defined. The aim of the present study was to evaluate interobserver and intraobserver variability in the description of bone graft fusion as viewed on standard x-rays of the lumbar spine.

Material and methods: In a prospective design 129 patients were randomized to PLF with CDI or without and evaluated after one year. The first 32 patients with CD and 36 patients without instrumentation comprised the material for this study. One consultant radiologist (Ra), 2 spine surgeons (S1&S2) and 2 registrars (Re1&Re2) rated the x-rays blindly two times, with a interval of 3 weeks. Intra- and interobserver variation were measured with Kappa statistic analysis.

Results: The mean intraobserver variation (Kappa-values): Ra=0.72, S1=0.45, S2=0.53, Re1=0.53 and Re2=0.59. All mean Kappa-values were classified as fair to good agreement. Complete bilateral union on the L5/S1 level was observed in 68% (mean, range 36%–77%) and on the L4/L5 level in 66% (39%–67%).

Conclusion: This study demonstrates the need for use of standardized criteria in the evaluation of PLF.

Changes in bone mineral after fusion in the lumbar spine with pedicle screw technique A longitudinal study with 1 year follow-up

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Introduction: The purpose of this study was to determine changes in bone mineral content (BMC) at the site of the posterior fusion grafts and changes in bone mineral density (BMD) above the level of fusion and in potentially stress shielded regions at the fusion level.

Material and methods: Bone mineral measurements were performed by Dual Energy X-ray Absorptiometry (DEXA).

Fourteen patients (F/M=8/6) who had a posterolateral fusion in the lumbar spine with pedicle screw technique and bone grafting, were scanned 7 days, 1H, 3, 6 and 12 months post-operatively. BMC was measured at the fusion graft site. BMD was measured above the fusion (L1), below the proximal screws (PS) and above the distal screws (DS).

Results: At the fusion graft site BMC decreased 64% within the first 6 weeks followed by an increase of 19% at 12 months follow-up. In the potentially stress shielded regions (PS and DS) BMD had decreased 3-4% at 12 months follow-up. In L1 a decrease of 3% was seen during the first 6 months followed by an increase of 2%.

Conclusion: The significant decrease at the graft site could be explained by a local resorption during the induction of new bone formation. The decrease seen in L1 followed by an increase may be the result of mobilization. Only a moderate decrease in BMD could be demonstrated in potentially stress shielded regions.

Bone mass and physical activity—comparison between loaded and unloaded skeletal regions

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The bone mineral density (BMD) was measured in 56 male and 33 female athletes, 18–40 years of age, as well as in 64 male ex-weight lifters, 35–79 years of age. The active athletes were compared with 95 age and gender-matched controls, the ex-weight lifters with 133 male controls. BMD was measured in the upper part of the skull, a region virtually unaffected by physical load, and in total body and femoral neck. The male athletes had a 3% higher BMD in the total body, 12% in the hip, whereas BMD in the upper part of the skull was 10% lower, as compared with the controls. Female athletes had a 4% higher total body BMD and a 10% higher hip BMD, whereas BMD of the upper part of the skull was 7% lower. Also, ex-weight lifters close to active career had higher bone mass in total body and femoral neck and lower BMD in the upper part of the skull as compared with controls. After 65 years of age no difference was found in any of the measured regions.

Conclusion: Active athletes have high bone mass in weight-loaded skeletal regions and low in unloaded regions. After exercising career these differences seem to be reduced.

Comparison of load transfer across normal and rheumatoid glenoids

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Introduction: Rheumatoid arthritis (RA) is known to have a profound effect on the bone quality near the affected joint. For the glenoid, it has been shown recently that the trabecular bone structure is severely damaged in patients suffering from RA in the shoulder. Both the presence of large cysts and a reduction in the size of the trabeculae result in a substantial reduction of the trabecular structure's stiffness and strength. How this affects the load transfer across the glenoid in comparison to a normal glenoid is the central question for this study.

Method: Load transfer was studied using a 3-dimensional finite element (FE) model of the glenoid. In this model (1,610 elements and 2,024 nodes), pathological bone conditions were simulated by a 90% reduction of the stiffness of the trabecular core of the glenoid and a 50% reduction of the stiffness of the cortical shell. For external loading, the glenohumeral (GH) joint force was simulated for abduction of the arm from 30 to 180 deg. (increment: 30 deg.).

Results: For the full range of abduction, the highest stresses were always found in the anterior part of the glenoid, whereby the locus of the stress peaks moved inferiorly with increasing abduction angle. In case of RA, higher stress levels and larger deformations were found throughout the whole glenoid as the subchondral plate is being pushed deeper into the glenoid. When merely the trabecular bone was affected by RA, stress levels rose up to 60% compared to the normal case. At the same time, the maximal inward displacement of the subchondral plate increased up to 163%. When the cortical bone was affected as well, these values were 45% and 343% respectively.

Conclusion: The FE analyses showed that a RA glenoid loses its normal ability to withstand the GH joint force. It is subjected to higher stresses and much larger deformations than a normal glenoid. In addition to the intrinsic loss of strength by the architectural changes of the trabecular structure, a RA glenoid thus becomes extra liable to fractures. Although not explicitly evaluated in the present study, this will also have implications for the placement of a total shoulder arthroplasty. As present glenoid components have a keel anchorage in the central glenoid, their stability in RA patients will also be impaired by the condition of the bone quality.

Kinematics of the lateral ligamentous constraints of the elbow

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Introduction: Only few reports on the lateral elbow joint stabilizers exists. The object of the present study was to evaluate the lateral ligamentous constraints of the elbow kinematically.

Material and methods: 28 osteoligamentous elbow joint specimens were included. Median age at death was 70 (47-86) years. The specimens were tested in an experimental stress apparatus. A small moment of 0.75 Nm. was used during stress, since this allowed repeated testings of the ligaments. Sequential ligament dissections were performed in the lateral collateral ligament (LCL), the annular ligament (AL) and the lateral ulnar collateral ligament (LUCL). These three structures constitutes the lateral collateral ligament complex (LCLC). Paired statistics were used, and results were significant when $p < 0.05$.

Results: Cutting the LCLC did not affect joint laxity during forced valgus and internal rotation. During varus stress separate sectioning of the LCL caused a max. varus laxity of 15.4° at 110° of joint flexion, whereas sectioning of the LUCL or the AL caused no laxity. Combined ligament cuttings induced max. varus laxity of 20°. Cutting the entire LCLC caused a max. varus laxity of 24°. During forced external rotation sectioning of the LCL caused a max. laxity of 23° at 110° of joint flexion. Separate sectioning of the LUCL or the AL caused no important rotatory laxity. Combined ligament cuttings caused a max. external rotatory laxity of 34°. Finally cutting the entire LCLC caused a max. rotatory laxity of 38°.

Conclusions: This study demonstrates the LCL as the most important isolated lateral ligamentous constraint of the elbow joint, resisting forces varus and external rotation. Further the LUCL was shown to contain fibers from the LCL. The LUCL was demonstrated not to induce any important stability to the elbow. Clinically this study shows that varus-, and rotatory elbow instability should be evaluated during elbow semiflexion between 70°-110°.

A sensory function of the posterior cruciate ligament

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Introduction: A muscular reflex elicited from mechanoreceptors in the ACL has been described, and probably plays a role for the normal knee function. The aim of this study was to clarify if the a muscular reflex is elicited when the sensory nerves of the posterior cruciate ligament (PCL) are stimulated.

Material and methods: In five patients, who were arthroscopied and who had no clinical or arthroscopic sign of PCL disease, two wires were inserted into the proximal and mid part of the PCL, respectively. When anesthesia had subsided, the PCL was stimulated with trains of electrical impulses.

EMG from muscles around the knee was measured during stimulation with the knee in full extension and 90 degrees flexion as well as under relaxed conditions and during active extension and flexion.

Results: During relaxation, a clearly visible excitatory reflex in the hamstrings muscles, especially biceps femoris was elicited. During active extension stimulation was followed by a strong inhibition of rectus femoris, medial head of quadriceps and gastrocnemius. During active flexion, the reflex was a strong inhibition of the hamstrings and gastrocnemius muscles with a latency of 80–100 ms.

Discussion: The length of latency shows that the muscular response elicited by stimulation of the sensory nerves of the PCL is of reflex origin and not voluntary. The long latency suggest that the reflex is polysynaptic or a long-loop reflex. It is unlikely that the reflex protects the knee from excessive strain by a direct synergy between the PCL and the muscles. The reflex could play a role in updating and formation of motorprogrammes in the normal knee function.

The PCL reflex demonstrated is nearly identical with the ACL reflex earlier described, except that the excitatory reaction is primarily in the biceps, whereas it is in the semitendinosus when ACL is stimulated. This can explain why some patients with ACL ruptures have a good proprioceptive knee function: If the ruptured ACL has healed to the PCL, the ACL stretch forces can be transmitted to the sensory nerves in PCL, reestablishing the ACL reflex to some extent.

The roles of implant motion and particulate polyethylene debris in the formation of an aggressive periprosthetic membrane

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Introduction: In the United States, over 20,000 revision hip replacements are performed annually. Particulate wear debris and implant motion are thought to play roles in aseptic loosening by stimulating the formation of an aggressive fibrous membrane which in turn secretes bone resorbing factors. This periprosthetic membrane is generally described as having synovial lining cells adjacent to the implant, dense, oriented fibrous tissue, macrophages, histiocytes and foreign body giant cells. The purpose of this study was to investigate the role of implant motion, with and without particulate polyethylene debris, in engendering the typical characteristics of a membrane surrounding aseptically loosened human implants.

Materials and methods: An unstable implant was used to represent a loosened human implant. It is a 6.0 mm diameter cylinder which pistons 500 μ m in the medial femoral condyle of a dog during each gait cycle. The magnitude and axial direction of motion during weight bearing is carefully controlled via implant design, and no external power source

is required. A loose cemented implant was modeled as a molded PMMA cylinder threaded on the unstable implant's core.

Eight mature mongrel dogs were randomized to two treatment groups: (a) implant motion alone, or (b) implant motion with particulate high density polyethylene (4.7 μ m mean (0.5–12 μ m); 100% pure). This particulate was intended to represent debris present in human retrieval studies. The particulate was ultrasonically and manually mixed with hyaluronic acid (LifeCore Biomedical, Chaska, MN) and injected in the implant cavity at a concentration of 0.5×10^8 particles/implant. The observation period was eight weeks.

Results: *Effect of implant motion alone.* All implants were surrounded by fibrous tissue with a reparative response with no inflammation. There was no neutrophilic, macrophage/histiocytic or lymphocytic response. Abundant vascularity was observed. *Effect of implant motion and particulate debris.* All implants were surrounded by fibrous tissue. Sheets of macrophages and histiocytes were present, often with intracellular PE particles. The membrane had synovial-like cells at the implant surface, and was composed of spindle shaped fibroblasts. PE particles were identified with polarized light and Oil Red O staining, and were seen to remain in the membrane surrounding the implant. PE was also seen in the adjacent bone. No foreign body giant cells were seen. Rare lymphocytes and cartilage formation were seen.

Discussion and conclusions: The results in this model of a cement-coated implant pistoning 500 μ m for eight weeks suggest that motion by itself was not enough to generate a membrane with the essential features of that retrieved from human revisions. The addition of particulate polyethylene (4.7 μ m mean diameter) to an unstable implant recruited macrophages, histiocytes, synovial cells and osteoclasts. Foreign body giant cells were not seen in this animal model. All other features characteristic of a membrane from an aseptically loosened human implant were present, however, with an unstable implant pistoning 500 μ m for eight weeks, in the presence of particulate polyethylene debris. These results suggest that in this model, particulate debris is necessary to generate an aggressive membrane as seen in clinical loosening.

Hydroxyapatite coating might prevent periimplant particle migration—a pilot study in dogs

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Introduction: Polyethylene (PE) debris is believed to be a major cause of aseptic loosening of joint replacements. Sealing off the bone/implant interface from the joint space seems therefore to be of great importance to prevent PE particle migration. Bobyne et al. (1995) have shown in a study of an unloaded press fit implant, that a porous implant surface is

more resistant to particle migration compared to a smooth implant surface. The aim of the study is to analyse whether a hydroxyapatite (HA) coating can prevent polyethylene particle migration compared to a non-coated blasted titanium surface.

Materials and methods: A loaded model based on the Søballe (1993) implant device was developed to test this hypothesis. Two implants were inserted into each knee of two bone mature dogs with a periimplant gap (0,75 mm), which communicated with the joint space. A HA coated and a non-coated implant (Ti-6-Al-4V, blasted surface) were inserted in each knee. Three weeks after surgery PE particles dispersed in sterile hyaluronic acid were injected into the right knee joint. The left knee served as control. The injections were repeated weekly, until the animals were sacrificed 8 weeks after surgery. Bone/implant specimens were obtained. The implants were gently removed after decalcification without damaging the bone/implant interface. Vertical sections of the interface were cut to identify particle migration along the implant. The specimens were stained with H & E and Oil Red O. The sections were viewed under polarized light with a light microscope.

Results: The non-coated implants were surrounded by a fibrous membrane and had no bone ingrowth. In contrast HA implants had bone ingrowth along the total implant surface. The interface from the non-coated implants showed many particles along the entire length of the implant. There were recruitment of macrophages and formation of foreign body giant cells around the particles. Interfaces from the HA implants showed a few particles located close to the joint space, but no particles at the base of the implant. No foreign body reaction was present.

Conclusions: This preliminary study indicates, that HA coating of implants may be able to prevent periimplant PE particle migration, by creating a seal, by enhanced bone ingrowth.

Significant resorption of hydroxyapatite and fluorapatite coatings on loaded implants

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Introduction: Resorption is a matter of concern for the long term fixation of ceramic coated implants. Several factors influence the resorption process, including different loading conditions. Fluorapatite (FA) was introduced as a ceramic coating (CC) to avoid the potential side effects of degradation of hydroxyapatite (HA) coating. The purpose of the present study was to estimate resorption of HA and FA coatings on loaded implants using a new stereological method, vertical sections.

Materials and methods: A stable implant device system, which allowed loading of the implant, was inserted bilaterally into the medial femoral condyles of 8 skeletally mature mongrel dogs. Porous coated, titanium alloy implants were plasma-sprayed with FA or HA. The dogs were killed after 25 weeks. Three serial vertical sections parallel to the long axis of the implant were ground to 50 µm to calculate blinded unbiased estimates. Non-inserted HA- and FA-coated implants served as controls. **Statistics:** Mean values were calculated. $P < 0.05$ was considered significant.

Results: Bone ingrowth to HA- and FA-coated implants was 60% for both implants types. The ceramic surface of the implants was covered by bone on 67 and 66 per cent for HA- and FA-coated implants, respectively. Marrow covered the remaining surface, apart from very small areas of fibrous tissue. The difference between bone and marrow ingrowth to the ceramic surface was significant for both CC's. The surface area of the implants covered with HA or FA was reduced significantly compared to controls by 18 and 13 per cent, respectively, but no difference between coatings ($p = 0.3$). Surfaces with complete resorption of HA were replaced by bone on 36 per cent of the surface, and by 29 per cent for FA coatings ($p = 0.4$). Volume and thickness of HA and FA coatings were reduced significantly, too, but no difference between coatings was found. Few CC particles were incorporated into surrounding bone close to the implant surface, but no foreign body reaction or osteolysis were found.

Discussion and Conclusion: HA and FA coatings were resorbed significantly, with no difference between coatings. Surfaces with CC had a higher coverage of bone than bone marrow compared to surfaces with complete resorption. Surfaces with complete resorption of ceramic coating was partly replaced by bone ingrowth, suggesting that the fixation of the implant will be maintained.

Transforming growth factor- β stimulates bone ongrowth to tricalcium phosphate coated implants

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Introduction: The growth factor *Transforming growth factor β* (TGF- β) has in numerous in vivo studies demonstrated stimulative effects on bone healing. Recent studies performed on dogs using ceramic coated implants and unloaded models, have also demonstrated stimulative effect of rhTGF- β 1 on bone ongrowth and mechanical fixation. In clinical practice a prosthesis component is weight loaded during each gait cycle. The aim of the present study was to investigate effects of rhTGF- β 1 adsorbed onto *tricalcium phosphate* (TCP) coated implants in a weight-loaded model.

Materials and methods: Weight-loaded cylindrical grit-blasted titanium implants (6x9 mm) coated with TCP ceramic were inserted into the weight bearing part of the medial

femoral condyles of 10 skeletally mature labrador dogs. The implants were initially surrounded by a 0.75 mm gap. In each animal an implant without growth factor and one implant with 0.3 µg rhTGF-β1 adsorbed onto the TCP coating was inserted randomly assigned to the right and the left knee. The dogs were sacrificed after 6 weeks. By histomorphometry bone ongrowth and bone volume in the gap was measured. Push-out test was performed to assess shear strength, shear stiffness and energy absorption on an Instron universal testing machine. Paired students T-test, P values less than 0.05 were considered significant. NS: nonsignificant. Mean and SEM in brackets is shown.

Results: All dogs completed the study. Bone ongrowth was significantly increased 59% by the 0.3 µg rhTGF-β1 stimulation (Table 1). Bone volume in the gap healing was increased 16% (NS). Mechanical testing did not show any differences between control and rhTGF-β1 stimulated implants (Table 1).

Conclusion: The present study demonstrated that rhTGF-β1 can increase bone ongrowth to Weight-loaded TCP coated implants in skeletally mature dogs. No improved mechanical fixation of the implants was found after 6 weeks observation. Growth factor stimulated bone healing could be an important concept in future cementless endoprosthetic surgery.

Table 1.	Control	0.3 µg rhTGF-β1
Ongrowth (%)	22.4 (5.6)	35.6 (2.9)*
Gap healing (%)	23.2 (1.6)	27.0 (3.0)
Shear strength (MPa)	0.67 (0.16)	0.55 (0.16)
Stiffness (MPa/mm)	5.73 (2.1)	4.17 (1.1)
Energy absorption (J/mm ²)	104 (31)	83 (24)

Method for demonstration of micro-vascular structures in human bone

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Introduction: Scanning electron microscopy (SEM) of vascular corrosion casts has been used for examination of vascular structures in bone. Until now, this has only been done in experimental animals. The purpose of this work was to apply techniques—improved in an animal study—for microvascular corrosion casting in human bone.

Materials and methods: Lumbar vertebrae (L₂) from autopsies were collected. The vertebral arch was removed and the lumbar arteries were catheterized. All lateral branches from the arteries except those supplying the vertebral body were carefully ligated before flushing with Ringer and injection

of methyl-methacrylate prepolymerized by ultraviolet light to a kinematic viscosity of 3.7 centiStokes. Four mm thick, vertical, frontal sections were sawed, macerated in 2% sodium-hypochlorite, cleaned in 5% Triton-X 100, rinsed in water, and dried, mounted on stubs, coated with platinum, and observed by SEM. Omission of decalcification allowed relations between the vascular system and the bone tissue to be studied.

Results: By this technique we were able to visualize for the first time the micro-vascular structures in human vertebrae and their relation to the trabeculae. We found close relations between resorption cavities and vascular structures with diameters from 10–50 µm, suggesting involvement of these vessels in the remodelling process. Vessels of the same size have been found entering or penetrating trabeculae. The findings support the theory of vascular involvement in remodelling in trabecular bone.

Conclusion: This method improves the possibilities for examination of the micro-vascular structures in human trabecular bone. It will be useful in studies of the vascular morphology in bone disorders.

Endothelial function in the isolated tibia after 10 hours of artificial perfusion

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Introduction: Acetylcholine induces endothelium-mediated vasorelaxation in the isolated, Ringer perfused tibia, from dogs (Moran, 1993) and rabbits (MacCarthy ARCO 1994). However, the response appears to be unstable and disappears within 4 to 6 hours of perfusion (Moran, 1992). Isolated resistance arteries from porcine bone exhibit little or no response to acetylcholine, although reproducible profound endothelium dependent relaxation to bradykinine is present (Lundgaard, trans. OAS 1993). The PURPOSE of this study was to evaluate the effect of acetylcholine and bradykinine in the isolated blood perfused porcine tibia and to study the stability of these endothelial functions with time.

Methods: Six 40 kg pigs were anesthetized, and the tibias were removed on the nutrient artery. After a short ischemic period (<5 min) the tibias were perfused with autologous heparinized blood via a pulsatile pump and placed in a bath with isotonic saline solution at 37 °C. Flow was regulated so as to give a bone perfusion pressure equal to blood pressure of the pig. After 3, 5, 7 and 10 hours bradykinine (10⁻⁶ M) and acetylcholine (10⁻⁵ M) were infused for 5 min. each, in random order. As control isotonic saline was infused at the same rate before and after the infusing periods. No precontraction was used because pilot infusion of norepinephrine produced irreversible contraction in this model. All measurement periods were preceded by 10 min steady state periods.

Results: Baseline bone perfusion pressure increased from AVG (SEM) 147 (18) mmHg after 3 hours to 183 (23) mmHg after 10 hours. We found a tendency to a decline in bone perfusion pressure during infusion of acetylcholine, this tendency, however, was not significant. After 3 and 5 hours there was no significant response to bradykinine but after 7 hours bradykinine produced a lowering of bone perfusion pressure from 173 (48) mmHg to 155 (40) mmHg, and after 10 hours from 181 (49) mmHg to 154 (43) mmHg ($p < 0.05$).

Conclusion: In this model acetylcholine does not produce relaxation of the arteries. This is in accordance with the findings by Lundgaard described above. Bradykinine produces relaxation after 7 and 10 hours indicating a preserved endothelial function.

Discussion: The increasing baseline perfusion pressure with time is probably due to local processes producing contraction. This may function as "precontraction" giving a response to bradykinine from 7 hours.

Endothelium-dependent relaxations in isolated nutrient artery segments of the rabbit femur

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Experiments were performed to investigate whether release of nitric oxide (NO) or an endothelium derived hyperpolarizing factor (EDHF) is responsible for the dilatation of the nutrient artery of the rabbit femur. Isolated ring segments of the femoral, communis and nutrient artery and the thoracic aorta of 6 rabbits were precontracted with phenylephrine (3×10^{-7} M). Subsequently, dose-response curves to acetylcholine (10^{-9} – 10^{-5} M) were made and repeated in the presence of tetrabutylammonium (3×10^{-4} M, an antagonist of EDHF) and N_v -nitro-L-arginine (L-NNA, 3×10^{-5} M, an inhibitor of NO synthase). Acetylcholine caused dose-dependent relaxations in all arteries. After administration of tetrabutylammonium, a significant decrease in the pD_2 -value was observed in the segments of the thoracic aorta (7.17 versus 6.73), the femoral (6.9 versus 6.2) and communis artery (6.8 versus 6.1) ($p < 0.05$). For the nutrient artery the decrease in pD_2 was statistically not significant ($p = 0.07$). The maximum relaxation was significantly altered in the communis artery only (91.5% versus 74.3%). L-NNA significantly decreased the pD_2 in the communis (6.89 versus 5.9) and nutrient artery (6.68 versus 6.15). The maximum relaxation was significantly attenuated in the thoracic aorta (78.6% versus 26.4%), the femoral (86.6% versus 21.4%) and the communis artery (91.48% versus 32.12%). The combined administration of tetrabutylammonium and L-NNA significantly decreased the maximum relaxation in all types of arteries and

this decrease was more pronounced than in the presence of L-NNA alone. In conclusion, both NO and EDHF appear to be involved in the acetylcholine induced relaxation of the bone nutrient vasculature. Contrary to our expectation, our data do not point to a dominant role for EDHF in the relaxation of the nutrient artery.

Effect of Gentacoll on epithelialization and neovascularization in excisional wounds

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The aim of this work was to analyze the effect of Gentacoll on the wound healing parameters: Epithelialization and neovascularization.

Material and methods: Standardized circular full thickness dermal wounds (D 2.25 mm) were created on the dorsum of each ear on 24 hairless homozygous mice. The cartilage layer was left intact. The wounds were treated with: a) $n = 17$, Gentacoll plates. b) $n = 15$, bovine collagen plates without gentamycin, and c) $n = 16$, Silicone film. The parameters were directly measured using intravital video microscopy and computerized planimetry. Measurements were performed immediately after wound creating and every third day until wound closure was achieved.

Results:

Days	Epithelialization mean \pm SEM	Neovascularization mean \pm SEM
Gentacoll	$n = 5$; $22.8 \pm 1.57^*$	$n = 0$
Collagen	$n = 11$; 14.5 ± 0.6	$n = 8$; 25.3 ± 0.7
Silicone	$n = 11$; 12.7 ± 0.7	$n = 10$; 25.1 ± 0.5

* $p < 0.01$ when compared with both control groups.

In the Gentacoll group only 5 out of 17 wounds reached complete epithelialization. Out of 12 wounds that did not epithelialize 9 were excluded because of inflammation followed by perforation of the cartilage layer and 2 were excluded because of inflammation followed by autodigestion. None of the Collagen or Silicone treated animals showed similar reactions.

Conclusion: This study showed that Gentacoll might damage exposed cartilage.

E- and L-selectin blockade in I/R injury in skin flaps

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E- and L-selectin are known to be partly responsible of the initial deceleration of neutrophils (PMNs) on the endothelial cells, prior to transmigration of PMNs out of the vessel during an inflammatory reaction. It has recently been shown that a shared antibody to E- and L-selectin (EL-246) enhances survival of myocutaneous flaps exposed to ischemia and reperfusion (I/R) injury. The benefit to the muscle component of the flap was of a much greater magnitude than to the skin. To further explore differences between pure skin flaps and myocutaneous flaps and to understand I/R injury in skin, we here study the effects of EL-246 on a pure skin flap model.

Methods and materials: Buttock skin flaps (10x18 cm) were created bilaterally in the pig (N=9). Flaps were subjected to 8 hrs. of ischemia and 20 hrs. of reperfusion. EL-246 (total dose=1 mg.) or saline was administered as a continuous direct intraarterial infusion just prior to reperfusion and during the entire reperfusion phase. Venous effluent from the flaps was collected for determination of L-selection on the PMNs by flowcytometry using Leu8-PE. Biopsies were taken for myeloperoxidase (MPO) measurements. Survival was estimated.

Results: There was no significant improvement in survival nor a decrease in MPO activity in the treated flaps. The percentage of L-selectin positive PMN was not decreased in placebo flaps as compared to systemic blood however, it was decreased in the treated flaps ($p < 0.05$).

Conclusions: The negative effect of EL-246 on survival and MPO activity further supports our suspicion of different mechanisms involved in I/R injury in a myocutaneous flap as compared to a pure skin flap. As a certain shear stress must be present for the selections to exert their effect, a possible explanation for the diverse results in muscle and skin might be caused by different reflow patterns. We are currently investigating this possibility.

Local heparin is superior to systemic heparin administration in preventing thrombus formation at the arterial anastomotic site in the rat

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Introduction: The aim of this blinded randomized experiment was to verify whether a topical application of unfractionated heparin (UFH) is equally efficient as a systemic bo-

lus of heparin in avoiding thrombus formation in arterial micro anastomoses.

Materials and methods: Three groups of 10 animals were treated intravenously (IV) with heparin (50 U/kg b.w., 100 U/kg, 200 U/KG) with the anastomotic site bathed in isotonic saline. A fourth group was treated with isotonic saline IV and locally with 100 U/mL heparin. The control group was treated with isotonic saline both IV and locally. To consistently reproduce thrombus formation an arteriotomy in the femoral artery which was closed by inverting part of the vessel wall into the lumen. To visualize thrombus formation directly the anastomotic site was transilluminated and recorded by in vivo microscopy for one hour. The two-dimensional area of the thrombus was subsequently measured with computer planimetry.

Results: The mean thrombus size in the control group was $0.24 \pm 0.14 \text{ mm}^2$. In the locally treated group mean area was $0.10 \pm 0.04 \text{ mm}^2$ ($p < 0.05$). In the 50 U/kg and 100 U/kg groups the thrombus sizes were $0.20 \pm 0.08 \text{ mm}^2$ and $0.18 \pm 0.08 \text{ mm}^2$, respectively. Both groups are significantly different from the locally treated group ($p < 0.05$). The thrombus size in the 200 U/kg group was $0.13 \pm 0.06 \text{ mm}^2$. There was excessive bleeding in the two groups treated with local heparin and IV heparin (200 U/kg) but no difference in blood loss. The APTT 2 hours after bolus injection was 51.5 sec (median) in the 200 U/kg group and significantly ($p < 0.05$) longer than the APTT in the control group (27.3 sec), the 50 U/kg (26.1 sec), and the local treatment group (23.4 sec).

Conclusion: Thus local heparin administration is superior to systemic administration due to less adverse effects.

Biochemical markers of bone metabolism and prediction of fracture

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Biochemical markers of bone metabolism have been evaluated in a population-based setting of 328 women aged 40-80 years. Bone mineral measurement by single photon absorptiometry (SPA) was undertaken, as was registration of retrospective and prospective fractures. Serum samples for analysis of Osteocalcin (OC), procollagen 1 carboxy-terminal extension peptide (P1CP) and carboxy-terminal telopeptide of type 1 collagen (ICTP) were taken.

OC was found to be 23% lower ($p = 0.006$) in women having sustained a fracture within 6 years prior to measurement after adjusting for age and BMC differences. P1CP and ICTP were not influenced by recent fracture history. In the oldest women, however, also P1CP was lower (11%, $p = 0.046$) in women who had sustained fracture.

OC and ICTP were positively correlated to age and negatively correlated to bone mineral content (BMC). The correlations of P1CP were weaker.

Prospectively, logistic regression gave an odds ratio (OR) of 1.8 ($p=0.015$) for a low P1CP and fracture susceptibility within 5 years and at a change of 1 SD independent of age and BMC. Comparing figures for a predictive value for fracture of a low 1CTP was 1.9 ($p=0.043$). OC was not predictive of fracture.

It seems as women who have sustained at least one fracture within recent years have an altered bone turnover with decreased bone formation but an unaltered resorption. A decrease from the mean of the collagen markers P1CP and 1CTP is associated with an increased risk for future fracture. P1CP and 1CTP have a similar influence on the risk of future fracture as forearm BMC (OR=1.6, $p=0.03$).

No effect of neuropeptides on human osteoblast metabolism and chemotaxis

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Introduction: Four different neuropeptides have been discovered in bone by immunohistochemistry; *calcitonin gene-related peptide* (CGRP), *vasoactive intestinal peptide* (VIP), *substance P* (SP), and *neuropeptide Y* (NPY). CGRP stimulates cAMP formation in osteoblast cultures (1) and inhibits PTH and PGE₂ induced calcium release from cultured bone, indicating inhibition of bone resorption (2). Furthermore, CGRP accumulates in experimentally fractured rat tibiae (3). VIP increases cAMP formation in osteoblast cultures (1) and stimulates osteoclastic bone resorption in vitro (4). CGRP, VIP, and SP are potent vasodilators (5,7), and NPY is a potent vasoconstrictor in bone (5). The purpose of the present study was to investigate possible effects of neuropeptides on human osteoblast metabolism and chemotaxis in vitro.

Material and methods: Osteoblasts were harvested from human trabecular iliac crest bone. At least 8 separate experiments with human osteoblasts from different individuals were investigated for each parameter. Cells in their second passage were used (6). The effect of the neuropeptides (*hVIP*, *hSP*, *hCGRP*, and *hNPY*, Peninsula Lab., England) on chemotaxis (10^{-11} – 10^{-6} M) and osteoblast metabolism (10^{-10} – 10^{-7} M) were studied. Data were expressed as ratios (mean \pm SEM) between stimulation and control values and compared to the responses of platelet-derived growth factor (PDGF) analyzed with t-test or ANOVA and Fishers LSD test.

Results: There were no significant effects of the neuropeptides on osteoblast metabolism. PDGF, but none of the neuropeptides, stimulated osteoblast migration.

Discussion: The lack of effect on human osteoblast metabolism of the neuropeptides may be ascribed to absence of stimulatory receptors and/or that simultaneous stimulation by other factors or hormones are necessary for biological activity of the neuropeptides in bone. The neuropeptides may exert their effect primarily on the hemodynamic regulation in bone, since the vascular effects in vitro and in vivo have been shown to be pronounced (5,7).

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Loss of bone mineral at the hip and proximal tibia assessed by DEXA following tibial shaft fractures

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Introduction: Posttraumatic loss of bone mineral in the peripheral skeleton following a tibial shaft fracture is well known.

Material and methods: Ten consecutive patients with a tibial shaft fracture were included in the study. Measurements of bone mineral were performed by DEXA (Norland XR-26 Mark II) within one week after the fracture and at follow-up after 3 and 6 months. BMD (d/cm²) was measured in both hips (femoral neck and greater trochanter) and in the lumbar spine L₂–L₄. BMC (g/cm) was measured bilaterally in the proximal tibia. Statistics: nonparametric two-way analysis of variance a.m. Friedman were used.

Results: During the first 6 months after a tibial shaft fracture, BMD in the hip decreased by 7.5% ($p=0.0012$) and 15.2% ($p<0.00005$) for respectively the femoral neck and greater trochanter. BMC in the proximal tibia decreased by 22.1% ($p=0.005$). The BMD of the lumbar spine was unchanged ($p=0.67$). The prospective changes in bone mineral of the healthy contralateral legs showed small, but non-significant decreases in the hip and tibia.

Discussion: Low BMD is an important risk factor for hip fractures in the elderly and posttraumatic osteopenia following a tibial shaft fracture might be a risk factor for later hip fracture.