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1. Intraneural edema and blood flow reductions in spinal nerve roots after short term epidural exposure to nucleus pulposus

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The aim of this study was to evaluate the possibility for epidurally applied nucleus pulposus to affect the endoneural space of spinal nerve roots after a short exposure time.

Material and methods: From 20 pigs, nucleus pulposus (NP) or fat was harvested. Vascular permeability: NP (n=5) or fat (n=5) was placed over the S2-S3 nerve roots for 1.5 h. Evans blue albumin (EBA) was then injected intravenously and allowed to circulate systemically for 30 minutes. Extravasation of EBA was assessed using fluorescence microscopy of nerve root sections. Blood flow: NP (n=5) or fat (n=5) was applied over the left S2 nerve root. Intraneural blood flow was then recorded during four hours, using the hydrogen washout method.

Results:

Table 1 Permeability

EBA	Number of nerve roots NP	Control
Mainly inside capillaries (0)	8	13
Focally among the axons (+)	3	4
Surrounding all the axons (++)	5	0

Edema (++) was found in specimens from four NP animals but in none of the controls.Blood flow was stable in all controls, whereas NP exposed nerve roots showed a mean value reduced by more than 50% after 3 hours.

Discussion: Nucleus pulposus application epidurally, resulted with a short latency in an intraneural edema and a blood flow reduction in nerve roots. These vascular changes may induce nerve root ischemia, which is intimately linked to nerve tissue injury and neuropathic pain. Intraneural vascular changes may thus be of pathophysiological importance in sciatica and nerve dysfunction.

2. The occurrence of sensory nerves in the interface membrane of aseptic loose hip prostheses

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The occurrence of sensory nerves in the interface membranes (n = 9) surrounding aseptic loosened hip prostheses was studied by immunohistochemistry. The analysis focused on substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) representing the sensory nervous system. Protein gene product (PGP) 9.5, a general marker of nerve fibres, was also analysed to establish the neuronal character of the SP-, NKA-, and CGRP-immunoreactive structures.

SP, NKA and CGRP-positive nerve fibres were identified in all samples. However, there was a difference in the density of nerve fibres both between and within the samples. There was also a difference in the distribution of sensory neuropeptides. SP- and NKA-positive fibres were predominantly non-vascular, forming varicose nerve terminals. CGRP-immunoreactive nerve fibres with varicose terminals were observed mostly in close proximity to blood vessels, but also as free nerve endings.

Sensory neuropeptides participate not only in nociception but also stimulate immune cells to release cytokines. Notably, cytokines are known to be involved in inflammation and bone resorption. The occurrence of sensory nerves immunoreactive to SP, NKA and CGRP in the interface membrane might reflect a pathophysiologic response contributing to aseptic loosening of hip prostheses.

3. Protective effect of the endothelin antagonist Bosentan on ischemic skeletal muscle necrosis

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The aim of this study was to test the hypothesis that the non-specific endothelin antogonist Bosentan improves postischaemic skeletal muscle reperfusion and reduces tissue damage.

Material and methods: 16 250–300 g male Wistar rats that underwent 3 h and 20 min of hindlimb tourniquet at 27 °C ischemia were included and divided in two groups. Perfusion was measured as Laser Doppler Flux (LDF) at 780 nm with continuous recording from a single fiber probe in the anterior tibial muscle during ischemia and the first 2 h of reperfusion. Blood pressure was measured in the carotid artery. The animals survived 72 h before histomorphometry of muscle cross sections. Groups were compared by Mann Whitney U-test. The treatment group received a Bosentan infusion of 15mg/kg before ischemia, repeated before removal of tourniquets, while the control group were given 0.9% NaCl solution.

Results: LDF recordings showed large spatial variations. The postischemic perfusion rate calculated as the minimum value of the initial 10 minutes, and the average of the LDF during the last 10 min of the observation period was higher in the treatment group ($p=0.015$ and $p=0.047$, respectively). Cross-sectional areas of necrosis were smaller in the treatment group ($p=0.002$), while the areas of no-reflow did not differ between the groups.

Conclusion: The study supported the hypothesis that Bosentan improves reperfusion and has a protective effect on postischemic skeletal muscle damage.

4. Reasons for variation of the rotational position of the femoral component in primary total hip replacement

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Malrotation of components is a predisposing factor for dislocation in total hip replacement (THR). Therefore, avoidance of extreme positions is recommended. The purposes of this study were to assess the variation in rotational position of the femur stem in uncomplicated primary THR, and to which extent the achieved rotational position and the change in rotation of each femur could be explained by the preoperative femoral neck anteversion.

Material and methods: Pre- and postoperative 3D-CT-measurements of the femoral neck anteversion in 43 patients operated with a primary THR.

Results: The mean postoperative femoral neck anteversion was 18° (SD: 9°, range 5°–51°) and not significantly different from the mean preoperative femoral neck anteversion ($P < 0.64$, paired t-test). The preoperative femoral neck anteversion could explain 60% of the total variation in rotational position of the femur stems. 7/43 patients had their rotation changed more than 10°. The changes in rotation conducted during surgery did not depend on the original femoral neck anteversion in the patient.

Conclusions: The rotational position of the femur stem was dependent on the femoral neck anteversion in the patient prior to surgery. There was no reduction in the range of femoral neck anteversion in this patient group.

5. Osteogenic Protein-1 Device® in combination with bone allograft and Pro-Osteon 200® around non-nemented implants

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Early incorporation of bank bone allograft is mandatory in revision of arthroplasties. We investigated, whether OP-1 (BMP-7) device (Stryker Biotech) further enhanced bone incorporation around implants and if Pro-Osteon 200 might be an alternative to bone allograft. OP-1 is a growth factor in the TGFß superfamily, which stimulates bone formation. Pro-Osteon 200 granules (Interpore, Irvine,US) are a corraline hydroxyapatite bone substitute with a microstructure similar to cortical bone.

Methods and materials: 6 labrador dogs had each 4 unloaded cylindrical hydroxyapatite coated implants inserted in the medial and lateral femoral condyles for 3 weeks. A 3 mm gap was left around each implant, which was filled according to the following groups: 1) allograft, 2) Pro-Osteon, 3) allograft + OP-1, and 4) Pro-Osteon + OP-1. The amount of Pro-Osteon, OP-1 and allograft was standardized by weight.

Results: Push-out testing showed that the OP-1 enhanced fixation of Pro-Osteon by 900%. No significant differences were found between Pro-Osteon + OP-1 and allograft with or without OP-1. No difference between allograft with or without OP-1 was found.

Conclusion: Implants treated with bone allograft were much stronger fixated than implants with Pro-Osteon alone. However, implants with Pro-Osteon combined with OP-1 were equally fixed compared with the allograft group.

6. Tensile bond between bone and titanium—a reappraisal of osseointegration

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When Brånemark in the 1970's established the term osseointegration, this implied a direct chemical bond between a titanium implant and bone. However, ultrastructural studies seemed not to support this idea, and osseointegration became defined by the absence of interfering fibrous tissue. Titanium was thus described as bioinert rather than bioactive. We now demonstrate mechanically a chemical bond between bone and titanium using unloaded c.p. titanium plates, similar to those used in previous studies on prosthetic loosening. Tensile force can only be transmitted by chemical bonds.

Materials and methods: Bone bonding was evaluated by a detachment test. The plates were developed such that a flat titanium surface came into contact with traumatized bone and that the rest of the detachable part had no contact with surrounding tissue. The titanium plates were either polished and sterilized in an autoclave, or treated in 4M NaOH and then heated to 600 °C according to Yan et al. (1996). After four weeks the plates were separated from the bone by a perpendicular traction force.

Results: The failure load of the untreated titanium plates never exceeded 0.03 MPa, whereas with treatment there was a force up to 0.78 MPa, with bone remaining attached to parts of the plates after detachment.

Conclusion: The present results confirm that a chemical bond is possible to obtain within 4 weeks with the described pre-treatment, and it may possibly occur also without treatment after a longer implantation time.

7. Impaction of cancellous bone grafts impairs osteoconduction

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The method of using morselized compacted cancellous allografts for hip arthroplasty revision shows results which seem to differ dramatically from other kinds of allografting. In structural cancellous allografts, bone ingrowth is usually limited to 2-3 mm, whereas morselized compacted grafts seem to be totally remodelled in several cases as judged by radiography. We compared impacted cancellous allografts with unimpacted. 17 rats had a Bone Conduction Chamber implanted in the tibiae bilaterally. On one side the chambers contained an impacted graft (bone volume fraction 65%), and on the contralateral side an unimpacted graft (bone volume fraction 35 %). Impaction of the grafts was done preoperatively with a pressure of either 25 or 2,500 MPa.

After 6 weeks the mean distance which the ingrown bone had reached into the graft was measured on histological slides. With both impaction pressures, the bone ingrowth distance was decreased to 30% of the unimpacted controls ($p=0.001$).

It appears that impaction alone does not have a favorable effect on the osteoconductive properties of a bone graft. On the contrary, impaction seems to disfavor osteoconduction. In the clinical situation, however, this is not necessarily a disadvantage.

8. Effects of hydroxyapatite coating and/or bone graft on the interfacial shear strength of primary and revision implants

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The decreased longevity of revision joint replacement implants reduces patient function, and increases health care costs. Our long-term goal is to determine methods to improve the results of revision implants.

Materials and methods: A micromotion implant in the canine medial femoral condyle modeled primary and revised implants. The revision setting was created by an 8-week implantation of an unstable PMMA implant, with polyethylene particulate (50% < 1 micron, range 0.5–50 microns) mixed with hyaluronic acid (0.5×10^8 particles/implant). After 8 weeks, the PMMA implant was removed, the cavity curretted, lavaged, and revised with a stable implant. At the same operative setting, an identical stable primary implant was installed in the opposite knee. The dogs were terminated 4 weeks later and results were evaluated by push-out test. Eight treatment groups (8 implants/group) evaluated stable porous Ti implants with or without HA, with or without allogenic bone graft, in primary and revision settings, in 32 mature mongrel dogs (64 knees).

Results and discussion: Fixation of revision implants was inferior to primary implants. In the primary and revision settings without bone graft, HA improved fixation strength over Ti. In the primary setting with bone graft, HA did not further improve fixation strength, whereas in the revision setting with bonegraft, HA did further improve fixation strength. With HA and bone graft, the fixation strength of revision implants was as high as for the best primary implants (i.e., either grafted Ti or HA). These results support further clinical evaluation of grafted HA implants in revision surgery.

9. Tumor necrosis factor-α induced leukocyte recruitment in lymphocyte function antigen-1 (CD11a/CD18) gene-deficient mice

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Leukocyte-endothelium interactions are key components in inflammatory conditions including arthritis and fracture healing. Leukocyte recruitment is a multistep process where leukocyte rolling is a precondition for subsequent firm adhesion. In vitro studies have shown that firm leukocyte adhesion is dependent on a group of heterodimeric molecules referred to as (2-integrins, including LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18) and p150,95 (CD11c/CD18). In the present study, we used intravital microscopy of the cremaster muscle in gene-targeted mice to examine the role of lymphocyte function antigen-1 (LFA-1, CD11a/CD18) in tumor necrosis factor-α (TNF-α)-induced leukocyte rolling and firm adhesion in venules in vivo. We found that 4 h intrascrotal administration of TNF-α (2000 units) markedly increased venular leukocyte adhesion. It was found that the increase in adhesion following TNF-α stimulation was similar in LFA-1 gene-deficient and wild-type mice. Moreover, the level of venular leukocyte rolling was almost identical in TNF-α and PBS treated mice irrespective of their genotype. In contrast, it was found that TNF-α stimulation markedly reduced venular leukocyte rolling velocity. Histological examination of hematoxylin-eosin stained whole-mount preparations revealed that more than 89% of the leukocytes in the TNF-α stimulated venules were granulocytes. Taken together, our functional data demonstrate that LFA-1 is not mediating TNF-(-induced granulocyte rolling and adhesion in mouse cremaster muscle venules in vivo.

10. A model to study human bone metabolism

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In order to perform further studies of the reaction of human bone metabolism we have developed a model for cultivation of human osteoblast-like cells. We have studied cellproliferation in order to obtain the optimal timimg for further experiments. The cells have been characterized to ensure that they are of a osteoblast-like type by the presence of alkaline phosphatase activity and osteocalcin production after stimulation with 1.25 (OH)2D3.

Materials and methods: Primary cultures of human osteoblast-like cells have been developed from human trabecular bone harvested from the femoral neck of patients with osteoartrosis undergoing total hip replacement. After removal of fat, cartilage and marrow the bone was cut into small fragments and ostoeblast-like cells were allowed to migrate from the bone pieces. The cells were grown to confluency within 6 to 12 weeks.

Results: We have found that the suitable timing for experimental work is 7–10 days after seeding the cells. All cell cultures produced osteocalcin which also was stimulated by 1,25 (OH)2D3 They were also positive for alkaline phosphatase staining.

Conclusion: Most studies on bone metabolism have been performed on cell lines from bone often with a malignant origin. We think that from a human physiological point of view it is more relevant to study human primary cells. We hereby demonstrate that this technique is a suitable model for such studies.

11. Corticotropin-releasing factor-like immunoreactivty in bone and joint tissue-a morphological and quantitative analysis in the rat

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The occurrence of corticotropin-releasing factor (CRF) was investigated in bone and joint tissues in the rat. The localisation of CRF in knee joint was assessed by immunohistochemistry (IHC) and the tissue concentrations were determined by radioimmunoassay (RIA).

CRF-positive nerve fibres were found in synovium, periosteum, bone marrow, ligaments, cortical bone and in trabeculae near the osteochondral junction. Interestingly, CRF-positive fibres were also identified close to chondrocytes in the articular cartilage. In general, abundant CRF-positive fibres were found close to the blood vessels.

Reproducible amounts of CRF was obtained in knee and ankle joints extracts by RIA. Similarly, CRF like immunoreactivity (LI) was measured in separate homogenates of cortical bone, periosteum and bone marrow. Higher CRF-concentration was found in the periosteum, bone marrow and ankle joints as compared to cortical bone and knee joints. The CRF-LI was characterised by reverse-phase high performance liquid chromatography

Previously, it has been postulated, that the main peripheral source of CRF is cells in the immune system. However, this study suggests, that the peripheral nervous system is another important source of CRF. It may prove that CRF participate in inflammatory pathophysiology and nociception by a neuronal mechanism. Furthermore, quantitative analysis of CRF in bone is possible by RIA.

12. OP-1 induces bone in healing tendon

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OP-1 is a growth and differentiation factor known to induce cartilage and bone. The differentiation of regenerating mesenchymal tissue is also governed by mechanical input upon differentiating cells. In a local environment which is mechanically directed at producing tendon tissue, it could be hypothesized that OP-1 would induce a tendon-like tissue.

We transsected the Achilles tendon in 66 rats. 25 of these also had the tibial nerve severed. This decreased tendon strength by 66% (p=0.0001) as with mechanical loading. OP-1 was applied to half of each group. Two weeks after the operation OP-1 had induced bone formation and further decreased strength in both groups (p=0.0001).

The results support the opinion that OP-1 in a mesenchymal environment generally is a bone inducer. This property overrode mechanical demand for a more traction-resistant tissue.

13. Detection of EWS/FLI-1 fusion protein by Western blotting and immunostaining—a diagnostic tool for Ewing's sarcoma and primitive neuroectodermal tumor

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Morphological diagnosis of ET (Ewing's sarcoma and PNET), based on biopsies or fine-needle aspirates, is often associated with severe difficulties. ET has been shown to carry the t(11;22) translocation in more than 90% of the cases. The fusion gene product has been shown to be a transcription factor.

Materials and methods: Using an antibody (C-19) against the C-terminal of the FLI-protein, Western blotting and immunostaining from ET-cells and tumors was performed.

Results: Western blotting showed a 68-kDa band which corresponds to the EWS/FLI-1 protein. This band did not appear in non-ET cells and tissues. Furthermore, immunostaining using C-19 from fine-needle aspirates and paraffin-embedded archival material from ET cases showed immunopositive cells. The signal was mainly confined to the cell nuclei, and was found to be specific since immunoreactivity was not seen when the control peptide was present in the antibody solution. Moreover, other types of small-round cell tumors were found to be negative. We could also confirm by Western blotting that non-ET-tumors and most normal tissues have none or a very low expression of wild-type.

Conclusion: Western blotting and immunostaining using an antibody against FLI-1 can be of diagnostic relevance in Ewing tumors carrying t (11;22).

14. In situ microdialysis in human bone tissue—stimulation of prostaglandin E2 release by weight-bearing loading but not by muscular exercise

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Enhanced prostanoid biosynthesis may be involved in anabolic events after mechanical loading. prostaglandin E_2 (PGE_2) is believed to play an important role in the recruitment of mesenchymal (osteoblastic) as well as osteoclastic cells in bone marrow. Here, we describe the first experiments using the microdialysis technique for studying the dynamic course of production of PGE_2 in the proximal tibia metaphysis in younger females, especially the effects of different kinds of mechanical loading.

16 healthy women, mean age 34 ± 2 years (± SD), with regularly menses and on no medication, participated. A standard microdialysis catheter was inserted in the proximal tibia metaphysis. Sampling was done every 15 minute. After a 2 hour equilibration period, heel-lifts with as hard impact of the heels into the floor as possible was performed by 6 of the subjects for 5 minutes, one per second while plantar and dorsal flexion of the foot was performed by 7 subjects for 5 minutes, one per second. Thereafter, sampling continued for another 2 hour period. Three subjects performed no exercise. PGE_2 was analyzed using a modified du Pont Prostaglandin E_2 RIA-kit.

Basal levels of PGE_2 in the cancelleous bone was determined to a mean of 45 (10 pg/ml and a 2.5–3.5 fold increase in PGE_2 was noticed secondary to weight-bearing mechanical loading. The increase was statistically significant ($p < 0.05$ compared to basal levels) one hour after the mechanical loading, and persisted for the rest of the experimental period. No major alterations in PGE_2 concentrations were observed in the group performing muscular exercise without impact loading nor the control group.

In situ microdialysis seems to be applicable for studying dynamic courses of synthesis of PGE_2 in human bone. Basal levels of PGE_2 in human bone was established and a significant increase in PGE_2 levels in the proximal tibia metaphysis of younger females was noticed after dynamic mechanical loading, while the production of PGE_2 was not increased secondary to non-weight-bearing muscular exercise.

15. Elevated IL-6 in osteo- and rheumatoid arthritic bone-derived osteoblast cells

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In an attempt to clarify the cellular basis of bone loss seen in rheumatoid arthritis (RA), it has been recently demonstrated that osteoclastogenesis is increased in bone marrow cell cultures from RA patients suggesting that the potential of increased bone resorption is constitutive at the cellular level1. Osteoclast-mediated bone resorption is essential in bone turnover and is partly osteoblast-regulated as osteoblasts produce osteoclast-stimulating factors such as interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin-6 (IL-6). The aim of this study was to investigate the constitutive expression of these cytokines in human osteoblast cells (HOB) derived from pathological bone.

Materials and methods: Bone was obtained from 7 patients with RA and 15 patients with osteoarthritis (OA) at the time of joint replacement. Normal (NO) bone was obtained from 6 patients following therapeutic amputation. Explants were cultured as described2 and first-passage cells were used in all experiments. Every third day, conditioned media were removed for protein quantification by ELISA and HOB were harvested for total RNA purification[3]. RNA were electroforesed on 1% agarose formaldehyde gels and transferred to Zeta-Probe membranes. Northern hybridizations were performed using human cDNA probes to IL-1 (α and ß), TNF (α and ß) and IL-6. Additional hybridizations with Histone 4 (H4), Collagen I (α) (COL I), Osteopontin (OP), Bone Sialoprotein (BSP) and Osteocalcin (OC) were performed to correlate cytokine expression to HOB developmental stages. Signals were quantitated by a Fuji Phosphorimager and normalized to a human 18S ribosomal cDNA.

Results: IL-6 mRNA levels were significantly higher in OA and RA HOB compared to NO. Maximal levels were reached (13-fold increase in RA and 5-fold increase in OA) at day 21 which corresponded to end of matrix maturation since COL I, OP and BSP mRNA levels were decreasing and OC mRNA levels were increasing. mRNA for IL-1 (α and ß) and TNF (α and ß) were undetectable. IL-6 protein secretion was significantly higher in OA and RA than in NO HOB at day 21 and 30. High levels of IL-6 protein had no significant effect on HOB proliferation and differentiation since H4 and phenotype genes were similarly expressed in all 3 groups.

Discussion: These results indicate that newly differentiated OA and RA HOB constitutively express and secrete high levels of IL-6 suggesting that they, independently of local inflammatory parameters, are able to stimulate osteoclasts and thereby bone resorption.

Our results support the recent observation that osteoclastogenesis is increased in bone marrow cell cultures from RA patients as IL-6 has been shown to promote osteoclastogenesis. Therefore, we suggest that the constitutive high IL-6 production by RA HOB stimulate very early osteoclast precursors resulting in an increased osteoclastogenesis in RA patients. Finally, our study confirm the pathogenetic role of IL-6 and make IL-6 an important target molecule for novel anti-cytokine therapy of arthritis.

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16. The effect of ovariectomy and ovarian steroid treatment on the tissue growth hormone and IGF-I in the rat

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Ovarian hormone deficiency is known to cause osteoporosis. We have previously shown that ovariectomy decreases the levels of growth hormone (GH) in the serum. In this study we investigated the effect of ovariectomy and ovarian steroid treatment on the tissue levels of GH and insulin-like growth factor-I (IGF-I) in the femur and bone marrow.

Materials and methods: 60 rats (9-month old) were ovariectomized (OVX) or sham-operated (SHAM). After 9 weeks 10 rats from each group were sacrificed and the rest were treated daily with s.c. injections of either 17b-estradiol (OVX-E), progesterone (OVX-P), or solvent vehicle only (OVX-V) for 10 weeks.

Results: Tissue levels of GH measured by specific radioimmunoassay method showed that ovariectomy decreased and estradiol (E_2) treatment increased the level of GH in the femur ($p < 0.05$). Similarly E_2 and progesterone treatment significantly increased IGF-I ($p < 0.05$).In the bone marrow, E_2 and progesterone treatment increased the GH levels ($p < 0.001$ and $p < 0.01$ respectively).

Conclusion: Our results suggest that ovarian steroids regulate GH and IGF-I levels in the serum as well as in the bone. We speculate that some effects of E_2 and progesterone on bone might be at least partially, mediated by GH and IGF-I.

17. Instability-induced bone resorption—effects of bisphosphonates

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We studied whether bisphosphonates can reduce bone resorption induced by an unstable implant.

Method: We used a rat model (J Bone Joint Surg 1996; 78-B: 641-6) in which a titanium plate is implanted on the tibia. The plate has a circular test surface, connected to a handle, which can be grabbed and turned through the skin of the rat, thus creating a sliding motion of the test surface against the bone.

At insertion, the bone was abraded with a burr. In order for a bone-titanium interface to be established, an interval of four weeks was allowed before movement of the plates was induced twice daily.

At the same time, alendronate 0.063 mg/kg/day, chlodronate 25 mg/kg/day or saline solution was administered either with osmotic minipumps or injections. After two weeks

of motion with or without biphosphonates, the rats were killed and histological sections of the bone surface were made.

Results: 1) Movement had induced bone resorption in controls and alendronate treated rats, decreased resorption was seen in chlodronate treated rats.

2) A significant increase in ashweight of the contra-lateral proximal tibia was seen in both bisphosphonate-treated groups, interpreted as evidence of a systemic effect on osteoclastic activity.

Conclusion: Chlodronate may be more useful for inhibition of instability-induced periprosthetic bone resorption than Alendronate. This resorption may be more difficult to inhibit than bone resorption as a part of normal remodeling.

18. Parameter identification of two different kinematic models of human joints

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We present a new method for fitting a specific model to the observed motion of a joint. Given data on the actual motion of the joint, by means of the 3-dimensional paths of a set of points, the parameters of the model are identified. The method is constrained to the use of two different models: 1) That of a rotational joint with a fixed axis of rotation, also referred to as a hinge-joint. And 2) a ball and socket model, corresponding to a spherical joint such as the shoulder.

The most important assumption is that the motion studied can be described adequately as that of a strictly rotational joint (hinge model) or a spherical joint (ball and socket model). The parameters of the models are in the case of the hinge model the five parameters necessary to describe the direction and position of the rotational axis. In the case of a ball and socket model, there are three parameters that describes the position of the center of rotation. By an optimization approach, parameters are found that give the "best" fit of the model to the observed motion. The optimization algorithms are linear, which eliminates the problems concerning convergence and ambiguous results that are common for non linear methods.

19. Early influence of an ankle sprain on objective measures of ankle joint function

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Outcome research of treatment modalities is often based on subjective measures such as questionnaires and scoring scales. These measures are difficult to interpret and to compare with other studies. The purpose of this study is to evaluate objective measures of ankle joint function.

Patients and methods: The study included 71 consecutive patients with an ankle sprain, seeking medical care within 24 hours from time of injury. The median age was 24 years (15–52). The patients were examined after 3–5 days, 2, 4 and 10 weeks after the injury with the following tests: eversion-inversion range of motion, recording of postural sway, joint position sense and isokinetic eversion-inversion muscle torque. In order to test the reproducibility of the postural sway and joint position sense test, 14 healthy female subjects were tested twice with three weeks interval.

Results and conclusion: A decreased range of motion, increased postural sway, a deficit in evertor muscle peak torque but no difference in joint position sense was observed during the follow up period in comparison with the uninjured ankle. In the healthy females the reproducibility of postural sway was good but the joint position sense test was not. We recommend these tests for evaluating ankle joint function with the possible exception of the joint position sense test.

20. Efficacy of arthroscopic lysis and lavage in patients with temporomandibular joint symptoms and generalized osteoarthritis or rheumatoid arthritis

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Lysis and lavage is the most widely used arthroscopic method when nonsurgical therapy has failed in patients with temporomandibular joint (TMJ) pain and dysfunction. No study on arthroscopic lysis and lavage in patients with generalized osteoarthritis (GOA) and TMJ symptoms has been published so far. Only limited information is available regarding arthroscopic TMJ surgery in patients with rheumatoid arthritis (RA) and TMJ symptoms.

Material: 23 patients with GOA and TMJ symptoms and 23 patients with RA and TMJ symptoms were included in the study.

Methods: Arthroscopic lysis and lavage of the symptomatic TMJ (in patients with bilateral symptoms was only the joint with most symptoms included) was performed under local anesthesia in the superior compartment. The patients were followed for 1 year.

Results: 17 RA patients improved after surgery, compared to 10 of the GOA patients. Lateral joint tenderness, crepitation, maximal opening and maximal protrusion were the variables that showed most improvement in the RA group.

Conclusion: Arthroscopic lysis and lavage seem to provide an effective treatment for TMJ pain and dysfunction in RA patients. In GOA patients, this seems more dubious.

21. Synovial inflammation in arthroscopically obtained biopsies in the temporomandibular joint—a proposed histologic grading system

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Data indicate that the synovial lining of the temporomandibular joint (TMJ) in some respects differ from other joints. The normal variation in the morphology of the synovial lining of the TMJ is quite large, whereas the variation in the pattern of pathologic changes appear to be relatively small (i.e. synovial inflammation is not to the severity of that of other joints). In the present review a system for histologic grading of synovial inflammation is proposed. The system is based on semiquantitative evaluation of the following set of parameters: 1) synovial lining cell layers, 2) vascularity (number and/or size of vascular profiles) and 3) inflammatory cell infiltrate (commonly lymphocytes).

22. Is increased glycosaminoglycan content corresponding to the high intratendinous signal in gadolinium contrast-enhanced magnetic resonance images seen in patients with chronic achillodynia?

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We evaluated the depicted tendon pathology in gadolinium contrast enhanced T1-weighted magnetic resonance images in chronic Achilles tendon disorder.

Materials and methods: 20 patients (16 men, 4 women, median age 40 years) with chronic achillodynia participated in the study. Clinical examination revealed swelling and tenderness localized to the mid-portion of the Achilles tendon. Contrast medium enhanced magnetic resonance imaging (CME-MRI) was performed in all patients. Ultrasound (US)-guided core biopsies were taken from regions with a clear widening of the tendon and a pathologic low echo signal as well as from normoechoic areas. Pathologic findings on US and CME-MRI were compared. Alcian blue/PAS stained specimens were analyzed with a histomorphometric method for quantification of glycosaminoglycan (GAG)-rich areas (GAG/collagen ratio).

Results: Histomorphometric quantification of GAG-rich areas in ultrasound (US) hypoechoic regions was 0.36 compared with 0.17 in normoechogenic regions ($p<0.001$). The volume of the intratendinous abnormality was larger in 13/20 when imaged by CME-MR ($p<0.05$), while the shape and enlargement of the tendon per se were similarly imaged by US and CME-MR.

Conclusion: CME-MRI revealed greater sensitivity in demonstrating intra-tendinous pathology than the US; this was documented by the larger size of the corresponding lesion and that the pathology was occurring in areas that were considered normal by US. The tendon pathology included increased content of water-retaining GAGs, which may correspond to the increased intratendinous signal following CME-MRI.

23. Cartilage induction by controlled mechanical stimulation

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In order to study mechanical control of tissue differentiation, we designed a new version of the previously described Bone Conduction Chamber (BCC). The BCC consists of a cylindrical titanium chamber implanted in the tibia of a rat. In the newly developed Load Chamber (LC), the top of the chamber has a mobile piston, so that an external compressive stress can be transferred to the tissue within the chamber.

Sprague Dawley rats had a regular BCC implanted in one leg and an LC in the other. Mesenchymal tissue was allowed to grow into the chamber for three weeks before starting the mechanical loading. Thereafter, twice a day, 20 cycles of 2 MPa compressive load were applied to the LC with a frequency of 0.17 Hz. The chambers were harvested after seven weeks of loading. All chambers contained new formed bone. The bone ingrowth distance into the chamber was decreased in the loaded specimens ($p=0.01$), but instead cartilage was found next to the piston. No cartilage was found in the unloaded controls.

In conclusion, we have developed a model for studying the differentiation of healing tissue during controlled mechanical stimulation, and found that a 2 Mpa compressive stress induced cartilage.

24. Collagen and fibronectin binding in experimental staphylococcal osteomyelitis

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One reason why osteomyelitis is difficult to treat is a decreased sensitivity to host defense mechanisms in microorganisms, that are adhered to implant surfaces or dead bone. Specific binding abilities between micoorganisms and host matrix proteins have previously been described. In a new mouse model of locally induced osteomyelitis we studied the pathogenic importance of specific binding ability to collagen or fibronectin in *Staphylococcus aureus.*

Materials and methods: A stainless steel wire was placed intramedullary in the right tibia of CBA mice. The animals were thereafter challenged with local inoculation of a S. aureus strain. For studies of the role of collagen adherence we used S. aureus strain Phillips, with collagen binding ability, and strain PH100, isogenic to strain Phillips except for lack of collagen binding ability. The animals used for collagen binding studies were inoculated with one of the two strains, or a mixture of both strains. For studies of fibronectin adherence S. aureus strain 8325-4, proficient in binding to fibronectin, and strain DU 5883 with inactivated fibronectin binding, were used. The animals were given single strain inoculations only. After one month the animals were killed and microbiologically evaluated.

Results and discussion: In contrast to previous studies in experimental arthritis models we found no differences in infection rates between the collagen and fibronectin binding, or non-binding strains. Despite the application of a foreign body, that decreases the number of microorganisms necessary to cause an infection, large amounts of bacteria were needed to induce reasonable infection rates. Such large inoculum sizes probably make specific adherence less important than in the clinical situation with small inoculi and low infection rates.

25. Insulin-like growth factor-I increases bone formation in old or corticosteroid treated rats

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Bone induction was studied in subcutaneous implants of demineralized bone matrix with or without insulin-like growth factor I (IGF-I) in aged or corticosteroid treated rats.

Each rat carried one pair of implants, one control and one experiment implant, containing IGF-I dissolved in a hyaluronan solution for slow release. The rats were killed after three weeks and the results were evaluated by measuring calcium content of implants.

Young (6–7 weeks) and old rats (19–27 months) were used. One group of young rats was treated for seven days with 140 µg/kg dexamethasone by daily subcutaneous injections.

Old rats produced only approximately 1 percent as much bone as young rats. Local delivery of IGF-I did not increase bone formation in young rats. In old rats, bone formation was increased by IGF-I at 3000 ng/implant. Corticosteroids reduced bone formation in young rats. This effect was partially reversed by local administration of IGF-I.

26. Proteoglycan turnover during development of spontaneous osteoarthrosis in guinea pigs

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In previous study, PGs losses seem to appear earlier than has been described for secondary OA. Our aim was to clarify the metabolic background of the variations in PG concentrations, relating to aging and the spontaneous development of OA in guinea pigs.

Materials and methods: 6-, 9- and 12- month-old Hartley guinea pigs were used in the experiments (n = 8). 35S was injected intraperitoneally. We analyzed the incorporation and elimination of various PGs in different areas of tibial articular cartilage during the development of OA.

Results: PG synthesis was most active in 6-month animals and highest in the medial compartment with its presumably higher load. The breakdown of large PGs decreased with age. The onset of OA was associated with decreased PGs synthesis, while the rate of elimination remained unchanged.

Conclusion: The decrease in PG contents at onset of primary OA is the result of a slow process involving only a slightly negative metabolic balance due to reduced synthesis and maintained degradation, quite different from secondary OA models, where an increase in the rate of degradation is more conspicuous. A higher load seems to reduce the ability of the chondrocytes to maintain the high synthetic rates, while the change in elimination is less obvious.

27. Immunoreactivity of osteopontin and CD44 in primary and metastasizing cancers

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Skeletal metastasis is one of the features of malignant diseases that causes the most morbidity and suffering. Osteopontin (OPN), a non-collagenous bone matrix protein, is involved in osteoclast adhesion and mineralization of bone. In many human cancers, high OPN expression is correlated with poor differentiation, invasive behaviour and high risk of tumor progression. Recently, a cell surface hyaluronan receptor (CD44) has been suggested to have a role in the mechanism of metastasis formation, possibly as a receptor for OPN.

Materials and methods: 26 tumor samples (15 breast and 11 prostatic cancers) and their respective bone and/or non-bone metastases were immunhistochemically investigated with polyclonal OPN antibody and with CD44 standard monoclonal antibody to assess expression of the markers by tumor cells in different tumor sites. Labeling was evaluated blindly and the number of labeled tumor cells counted at the light microscopic level.

Results: Strong OPN expression, ie >50% labeled cells, was found in about half of the primary tumors. In ductal breast cancer, almost all metastatic bone tumors showed strong OPN expression. Prostatic cancer exhibited an reverse pattern. Interestingly, in both breast and prostatic cancer, labeling was invariably stronger at the periphery of the tumors. In breast cancer, lobular and ductal cancers had different patterns of OPN expression.

Table.1 Pattern of strong osteopontin immunolabeling

Site	n	Primary	Bone	Other
Breast	15	6 (40%)	11 (92%)	2 (20%)
Prostate	11	5 (45%)	2 (20%)	1 (50%)

Table 2. Pattern of strong CD44 immunolabeling

Site	Primary	Bone	Other
Breast	10 (67%)	3 (25%)	5 (50%)
Prostate	10 (91%)	3 (30%)	0

Conclusion: In human cancer OPN expression has previously been associated with dedifferentation and progression. Our data suggest that OPN may also be involved in the process of breast cancer metastasis.

28. Neurokinin-A in bone and joint tissues—changes in adjuvant arthritis

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Adjuvant arthritis is a chronic inflammatory experimental disease in the rats which resembles rheumatoid arthritis in humans. Clinical and experimental observations suggest that the nervous system is involved in the pathophysiology of inflammatory joint disease. Tachykinins, a structurally related family of neuropeptides, a common precursor for SP and neurokinin A has been identified. They merits consideration as a potential contributor to arthritis.

Material and methods: The localization of neurokinin A (NK-A) in normal ankle joint of rat was investigated employing immunoelectron microscopy. Using radioimmunoassay the concentration of NK-A was investigated in bone marrow, synovial, cortical bone and ankle. Chronic adjuvant arthritis induced by intradermal injection of heat killed Mycobacterium butyricum.

Results: Immunoelectron microscopy showed immunoreactivity of NK-A in the myelinated nerve fiber in the periosteum. Detectable concentration of NK-A was observed in bone marrow, periosteum, cortical bone and ankle of normal rats. The concentrations of NK-A in arthritic ankles and spinal cords were found to be significantly increased compared to control rats.

Conclusion: These findings indicate the existence as well as a biological role of NK-A in bone and joint tissues. It could be speculated that inhibition of NK-A might alleviate the inflammatory and nociceptive symptoms in arthritic diseases.