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Roentgen stereophotogrammetric analysis of the Mallory Head (Biomet, Warsaw, USA) prosthesis without markers

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Micromovements of prostheses can be measured accurately by means of Roentgen Stereophotogrammetric Analysis (RSA). To this purpose, the prosthesis and the surrounding bone are marked with tiny metal pellets. The marking of the prosthesis, in particular, is regarded as disadvantageous. For this reason, for the Mallory Head hip prosthesis an adapted RSA method was developed, which requires no marking of the prosthesis but which uses prominent points of the prosthesis itself. A study was carried out in order to establish if the adapted RSA method is accurate enough for the measurement of micromovements of the Mallory Head hip prosthesis.

Material and method: With the adapted RSA method, the spatial position of the prosthesis is determined using measuring points on the head, the stem and the tip of the prosthesis. To test the method, RSA photographs were made of a phantom placed in nine different positions. The phantom consisted of the femoral component of the Mallory Head hip prosthesis glued in a perspex cylinder. A number of tantalum markers were placed on the outline of the cylinder. The measurements of the positions of the markers and of the prominent measuring points of the prosthesis in the photograph were carried out using an accurate measuring table (Zeiss Planicomp 100). The calculated position of the cylinder in space was the golden standard with which the position of the prosthesis was compared.

Results: As the prosthesis was bonded inside the cylinder with glue, no relative movement between the prosthesis and the cylinder proved possible. However, relative movements were nevertheless measured as the result of measuring errors. The magnitude of these relative movements was a measure of the magnitude of the measuring error. The relative movements of the prosthesis in relation to the cylinder were determined in eight cases, and expressed in spiral axis parameters. Two of the spiral axis parameters were the translation along and rotation round the spiral axis. The mean relative translation amounted to 0.005 mm, the mean relative rotation to 0.17 degrees. The 95% confidence intervals of these two parameters ranged from -0.04 to 0.05 mm and from 0.00 to 0.36 degrees, respectively.

Conclusion: The newly developed adapted RSA method proved to be accurate enough to determine the micromovements of the Mallory Head prosthesis. The exactitude of measurements in patients still remains to be investigated. In practice, the image quality will probably be less than that obtained with the measurements in this experimental set-up. It is expected, however, that measurements with the adapted RSA technique in vivo will have an exactitude comparable with that of RSA measurements with pellet-marked prostheses.

The interaction of a scoliotic spinal column and a corrective memory rod (a simulation model)

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The department of orthopedics of Groningen University Hospital is engaged in the development of an implant made of memory metal for correction of scoliosis. A study was performed with the objective to design a serviceable simulation model of the mechanical behavior of the linked system of memory rod and scoliotic spine.

Material and method: The simulation model is inspired by the following clinical technique. An originally straight rod is bent into such a shape that it can be linked to a number of vertebrae. After heating of the rod to above a certain temperature, it will tend to regain its original shape. In the process, the rod exerts forces and moments on the spine that will (partially) correct the deviation. Owing to the visco-elastic qualities of the spinal column, this process will take place gradually over a certain length of time.

The simulation model is based on the following hypotheses:

1) The spinal column is modelled as a chain of vertebrae with intervertebral discs between them. It is mostly these intervertebral discs that are responsible for the visco-elas-

tic behavior of the spinal column.Y
2) The memory rod is regarded as a continuous bending rod.
3) The behavior of the memory rod is described by assuming a particular correlation between the internal moment and the local curvature.
4) A number of anchors connect the rod with the spinal column and transfer the forces and moments from the rod to the spinal column and vice versa.

The calculations of the simulation model are adapted to these hypotheses and to the kinematics of the linked system, opting for finite displacements and ditto rotations according to Euler.

Results: The simulation model meanwhile has been realized on the basis of the above–mentioned hypotheses, with the result that in the model the rod and the spinal column converge toward a stable spinal situation.

After halving of the mesh size between the discreticized points on the memory rod and halving of the discreticized time step, the ratios of the errors in the infinite norm are found to be four and two, respectively. This is in accordance with the analysis of the numerical convergence.

Discussion: The validity of the numerical test calculations is limited because available information on the physical relationships of the spinal column and the memory rod is still insufficient. Nevertheless, the simulation model represents a first step on the way to clinical calculation and application in the preoperative planning in scoliosis patients. Work on a refined programming of the model is in progress.

In-vivo contraction patterns of the rotator cuff of the shoulder muscles

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The muscles of the rotator cuff, because of their particular location and configuration, play a major part in the stabilization of the glenohumeral articulation. Experimental data on the in-vivo functioning of the rotator cuff muscles are scarce, however. The contraction pattern of these muscles was studied in test subjects with the aid of electromyography.

Material and method: In healthy volunteers, EMGs were prepared, by means of wire electrodes, of, in particular, the supraspinatus and infraspinatus muscles (n=6) and of the three parts of the subscapular muscle (n=6). The EMG activitiy of the shoulder muscles was related to the direction of the external force exerted by the test subject.

Results: Plotting of the EMG activity against the direction of the force exerted provided typical activation patterns for most muscles: a flat, inactive part and a parabolic active part, with a distinct peak activity. Of the rotator cuff muscles, the supraspinatus muscle and the farthest caudal portion of the subscapular muscle also displayed similar patterns. The infraspinatus muscle, on the other hand, was active whatever the direction of the force. The same applied to the cranial portion of the subscapular muscle.

Discussion: An activation pattern such as that of the infraspinatus muscle appeared to correspond best to the function of stabilizing muscle. The fact that not all muscles of the rotator cuff displayed such an activity suggests that the rotator cuff should not be regarded as just one functional unit, but probably combines stabilization tasks with primarily 'moving' tasks. In the future, a comparison of the EMG activity patterns with the moment arms obtained from a three-dimensional dynamic model of the shoulder and implemented in dynamic experiments, may provide more insight into the complex activity of the rotator cuff; the expected finding is that the demands made on the stabilization of the glenohumeral articulation will be the higher ones.

Multiple epiphyseal dysplasia—a clinical diagnosis reduced to a collagen IX abnormality

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Osteochondrodysplasias formerly constituted a highly heterogeneous group of diseases, difficult to bring together under one common denominator. They are hereditary diseases. Currently, it is known about a large number of these abnormalities via what chromosome and via what gene they are inherited. For instance, it is known about a number of osteochondrodysplasias that a mutation is involved in the gene that codes for a collagen chain. With regard to multiple epiphyseal dysplasia, two possible loci are known: one on chromosome 1 and one on chromosome 19. An investigation was conducted to detect the gene that might be responsible for the heredity of this type of epiphyseal dysplasia. Subsequently, the question was studied what consequences the abnormal gene might have for the collagen IX alpha-2 chain.

Material and method: A family with multiple epiphyseal dysplasia was re-examined for the purpose of this study and wherever possible, radiographs were made of all patients with signs or symptoms. Subsequently, an extensive genealogy was drawn up. For the search for the abnormal gene use was made of DNA from venous blood and several markers from the socalled EDM1 and EDM2 regions.

For the COL 9A2 mutation, use was made of chondrocytes and lymphoblasts of one female patient. DNA was increased with the aid of PCR reactions; the defect was investigated during a further analysis.

Results: The EDM1 locus on chromosome 19 was excluded. For the EDM2 locus, a significant correspondence was established; the maximal lod score was 15.31 with a recombination factor of 0.016.

In the COL 9A2 mRNA, a defect of 36 nucleotides was observed, resulting in a defect of 12 amino acids in the alpha-2 collagen polypeptide.

Discussion: The results of this study strongly suggest that the abnormal gene in the examined family with multiple epiphyseal dysplasia is to be found on chromosome 1. The defect in the collagen IX alpha-2 chain of 12 amino acids probably results in an insufficient collagen IX. This might constitute a possible explanation of the fact that the abnormalities in multiple epiphyseal dysplasia occur in the cartilage of the epiphysis.

Bone resorption in the distal femur after implantation of a total knee prosthesis

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When a revision operation of the femoral component of a total knee prosthesis (TKP) is necessary, the amount of bone present is often found to be insufficient. Radiographic examination and DEXA measurements have demonstrated that bone resorption in the distal femur indeed occurs frequently, especially behind the anterior flange of the prosthesis. Finite elements (FE) analyses show that the mechanical stresses in the distal femur are changed by the implantation of a TKP resulting in stress shielding. An investigation was performed in order to determine if the bone resorption encountered can be attributed to stress shielding and if the characteristics of the bonding between prosthesis and bone may exert influence on the preservation of the bone mass.

Material and methods: A FE model was made of an average femur. In addition, a model was made of the same femur to which the femoral component of a knee prosthesis with intercondylar box was applied. Of the latter model, two different FE analyses were made: one with a completely fixed interface between the prosthesis and the bone cement, and one with a completely loose interface.

The long-term prediction of the bone density was based on a stretch-adaptive bone remodelling theory. This theory assumes that bone cells react to local changes in stretch energy density (SED). The degree of bone modelling was determined by calculating the time-dependent change in the bone mineral density (BMD). To this purpose, simulated DEXA scans were made in the frontal and sagittal planes.

Results: The remodelling simulations predicted an enormous loss of bone in the distal femur. It could be deduced from the sagittal scans that pronounced changes occurred in the BMD at the front of the distal femur. At the distal end, the BMD decreased by 94% in the analysis of the fixed prosthesis and by 63% in that of the loose prosthesis. An increase of the BMD was observed at the level of the proximal end of the anterior flange of the prosthesis; far proximally in the posterior portion of the condyles, some bone formation was found in the case of a fixed prosthesis. Distally, however, there was bone loss and these two together resulted in a decrease of the BMD by 9%. The analysis of the loose prosthesis in this respect revealed both distal and proximal bone loss with a mean decrease of 27%.

It could be deduced from the frontal scans that the BMD in the presence of an adequately fixed prosthesis decreased in the distal portions of the medial and lateral condyles by 68% and 93%, respectively, while in the case of a loose prosthesis, these losses amounted to 38% and 86%, respectively.

Discussion: The analyses of the fixed and loose femoral components may be regarded as two extreme situations for the alteration in BMD after implantation of a TKP. In two other, roentgenological studies, it was reported that bone loss in the anterior portion of the distal femur occurred in approximately 70% of the patients with a TKP who were examined. It should be pointed out that in these patients no DEXA measurements were carried out.

The bonding between prosthesis and bone has significant effects on the long-term evolution of BMD. When the analysis of the loose prosthesis is compared with that of the fixed prosthesis, it is found that a well-fixed femoral component results in higher BMD values when a situation of equilibrium is reached in which the decrease of bone proceeds more slowly.

The bone loss beneath the femoral component of a knee prosthesis may be attributed to stress shielding; the amount of bone lost may be quite large. Possibly, this bone loss might be limited by means of an optimalized design of knee prosthesis and/or improved fixation characteristics.

Torsion of the bone-patella-bone transplant in reconstruction of the anterior cruciate ligament improves the stability of the knee

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Isometric anterior cruciate ligament (ACL) reconstructions with a bone–patellar–bone (BPB) graft are sometimes performed with a twist (axial rotation) in the ligament. The effects of this twist in the transplant on the stability of the knee are unknown, however. For this reason, a study was carried out with three knee preparations, measuring the influence of the torsion of a BPB transplant on the anterior stability.

Material and methods: In three intact knee preparations, the anterior drawer phenomenon was measured in 0, 15, 30, 60, 90 degrees of flexion with 100 N anterior load. Subsequently, the drawer phenomenon was measured after severing of the anterior cruciate ligament and finally, measurements were carried out after isometric reconstructions had been performed with the BPB transplant in torsions of 0, 90 and 150 degrees (exotorsion on the tibial side). The displacements of the tibia were measured using Roentgen Stereophotogrammetric Analysis. The femoral bone plug of the graft was fixed with an 'interference screw' (a screw that wedges the bone plate tight in the femoral tunnel). A cylinder was

placed in the tibia in which the tibial bone plug could be fixed in every rotation position with application of pre-stress; the pre-stress each time amounted to 20 N and was applied in a position of 20 degrees of flexion.

Results: The findings obtained in this study showed for each of the three knee preparations that:the anterior drawer phenomenon of the intact preparations was maximal in 20 degrees of flexion; the drawer phenomenon increases distinctly after severing of the ACL, especially in 20 degrees of flexion (8 mm for preparations I and III, and 6 mm for preparation II); reconstruction of the ACL with the BPB graft substantially improves the stability of the knee as compared with the situation without ACL, but normal values are never regained; reconstruction with outward torsion of the BPB graft results in an antero–posterior stability that is more like the normal situation than reconstruction without torsion.

Discussion: Outward axial rotation of the BPB graft in ACL reconstructions slightly improves the stability of the knee as compared to a reconstruction without rotation. Possibly, this effect is due to improved fiber orientation in the antero-posterior direction and improved bundling of forces of the fibers in the transplant.

Effect of osteogenetic protein-1 (OP-1) on human bone marrow cells in vitro

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OP-1 (bone morphogenetic protein 7) is a member of the TGFa 'superfamily' and, when implanted subcutaneously in mice, can induce enchondral bone formation.

In vitro, OP-1 stimulates the osteoblastic phenotype in osteoblast-like cells of various laboratory animals. As a part of research into biological bone replacement, the effect of OP-1 on human bone marrow cells was studied.

Material and method: The alkaline phosphatase (AP) activity and the osteocalcine production were measured as markers of osteogenic differentiation. The experiments were carried out with the mononuclear fraction of bone marrow from the iliac crest; cells obtained in this manner were cultured according to two different methods. In the first place (method 1), the cells were first pre-cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum and antibiotics at 37 °C and 5% CO_2. Subconfluent secondary cultures were stimulated for three days with 0 or 30 ng/ml OP-1. Subsequently, the cell layer and the conditioned medium were collected. With method 2, the cells were cultured in the above-mentioned medium to which 0 or 40 ng/ml OP-1 was added immediately. The cells were cultured in this medium until confluence occurred.

Results: In the cell cultures from method 1, the AP activity had decreased significantly to 0.6 times the control value (n=4, SD=0.3, p=0.05). The cells cultured according to method 2, on the other hand, showed a significant increase of the AP activity to 3.7 times the control value (n=3, SD=1.6, p=0.05). The production of osteocalcine, measured in the conditioned medium, showed no increase in either group. It could be shown by means of this study that cells from human bone marrow have the capacity to respond to OP-1 with an increase of an early osteogenic parameter: the AP activity. A later characteristic of osteoblastic phenotypes, the production of osteocalcine, was not observed. Possibly, OP-1 induces osteogenic differentiation while other factors are necessary for further maturation. Neither marker was found in cell cultures prepared according to method 2. Possibly, in these cultures, the target cells of OP-1 have disappeared. It was concluded from this study that OP-1 can provide an osteoinduction of cells from the bone marrow which disappear from the culturing system if OP-1 is not added immediately.

This finding suggests that human bone marrow in vitro responds to OP-1 with osteoinduction and that OP-1 possibly may stimulate the bone formation in bone-replacing operations.

Osteogenetic protein (OP-1) as an anabolic agent for regeneration of cartilage

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The recently discovered fact that bone morphogenetic proteins (BPMs) can induce enchondral bone formation in adult animals suggests that BMPs are very closely involved in the formation of both cartilage and bone. It has been demonstrated that recombinant human osteogenic protein-1 (hOP-1 or BMP-7) can repair large segmental defects in the diaphysis of non-human primates. This indicates a high therapeutic potential of hOP-1 as an anabolic agent for the repair of non-unions, levelling up of alveolar bone, spondylodesis and the promotion of osseous integration in the fixation of prostheses. The in-vitro findings further suggest that OP-1 might exert a stimulating effect on the formation of cartilage after an articular injury. For these reasons we studied the effect of OP-1 on the production of cartilaginous matrix in cultures of perichondrium and periosteum.

Material and method: Primary cell cultures were obtained from perichondrial biopsy samples from ribs and ears of adult female pigs and compared with costal periosteum. Perichondrium and periosteal cells were obtained from proliferation of tissue fragments. The cells formed a confluent cell layer in 19 days. Subsequently, $25x10^3$ cells were cultured as socalled micromass cultures in culture medium DMEM with 2% fetal bovine serum for 5 days without or with hOP-1

(0.4–40 ng/ml). For determination of the rate of the biosynthesis of the proteoglycan, the cells cultured were labelled with 10 μCi/ml [35S] sulphate for 24 hours.

The non-incorporated [35S] sulphate was removed, and the numbers of labelled macromolecules of the cell extracts were determined.

Results: In perichondrial cells, hOP-1 stimulated the incorporation of [35S] sulphate in a dose-dependent manner. The stimulation by hOP-1 reached a maximum of 62% at 40 ng/ml. No effect of hOP-1 was observed, however, on the incorporation of [35S] sulphate into periosteal cells.

Conclusion: The stimulation of the proteoglycan synthesis by hOP-1 in perichondral but not in periosteal cells implies that this response is specific of the perichondrial cells. This study demonstrates that hOP-1 exerts a direct stimulating effect on the production of cartilaginous matrix for perichondrial cells in vitro. This might be the cellular mechanism of the induction of cartilage formation by hOP-1 in vivo. In other words, our results show that hOP-1, because of its exceptionally strong effect on the synthesis of proteoglycans, might exert a stimulating effect during the earliest phases of cartilage formation. Consequently, in orthopedic surgery hOP-1 might represent a highly interesting option for the regeneration of cartilage in cartilaginous defects in joints.

Detection and quantification of collagen expression in human articular chondrocytes by means of RT-PCR and in situ hybridization

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The capacity of recovery of articular cartilage is limited. Large defects will result in early osteoarthrosis. One of the latest techniques to repair articular cartilage is autotransplantation of perichondrium. An essential factor for functional recovery of the cartilage is the differentiation of the mesenchymal stem cells present in the perichondrium into mature chondrocytes. This differentiation is accompanied by a change in the collagen expression. Stem cells synthetize collagen type I, while mature chondrocytes synthetize collagen type II. Inadequate regulation of this differentiation may cause chondrocytes to dedifferentiate (once more bring collagen type II to expression) or hypertrophy (bring collagen type X to expression). In the latter case, the cartilage undergoes calcification. The present study had as its objective to determine if the three stages of chondrocyte differentiation occur in a culture model of human articular chondrocytes and if the cells can change from one stage of differentiation to another during the culturing. As markers for the three differentiation stages (prechondrocyte, mature chondrocyte and hypertrophic chondrocyte) were used the expressions of collagen type I, type II and type X, respectively, determined with the aid of molecular biological techniques.

Material and methods: Articular cartilage was collected in the operation room during implantation of a hip or knee prosthesis. Chondrocytes were isolated after collagenase digestion of the cartilage for 17 hours. Cell cultures consisted of 300.000 cells in 1 ml culture medium; the cultures were maintained for 5 to 24 days in a culturing incubator. After harvesting of the cells, total RNA was isolated from 10-15 cultures, or cultures were fixed in 4% formaldehyde for 24 hours and imbedded in paraffin. Total RNA was digested with RNAse-free DNAse to remove remnants of genomic DNA. Reverse transcription of RNA was carried out with Moloney Murine Leukemia Virus reverse transcriptase and random primers.

PCR was carried out with primers for COL10A1, for COL2A1 and for COL1A1.

An internal standard containing the same primer sequences and a fragment of ovalbumin gen was constructed and used for the quantification of the amounts of mRNA of the various collagens.

In situ hybridization was carried out with [35S]-labelled RNA probes for the collagen alpha1 chain of collagen type I, collagen type II and collagen type X (a present from Dr T. Aigner (1)). A probe for GAPDH and treatment of the section with RNAse served as the positive and negative controls, respectively. Results

Human chondrocytes isolated from arthrotic articular cartilage and cultured in high density, induce expression of each of the three collagen types. This appeared from the results of both the RT-PCR and the in situ hybridization. It was a general finding that with the duration of culturing, the amounts of mRNA of collagen types I and X increased and the amount of mRNA of collagen type II decreased. It appeared from the in situ hybridization that the cells in the center of the cultures induced expression of collagen II and sometimes also, of collagen X, while the cells at the edges of the cultures mostly produced collagen type I mRNA. Histological examinations and in situ hybridization with the GAPDH probe demonstrated that the viability of the cells decreased with increasing duration of culturing.

Conclusion: This culturing model of human chondrocytes from arthrotic articular cartilage proved to contain chondrocytes in three different stages of differentiation side by side. Some chondrocytes induced expression of collagen type I, other ones of collagen type II and still other ones, of collagen type X. The cells in the centers of the cultures were largely surrounded by newly synthetized extracellular matrix and partly for this reason displayed the chondrocytic phenotype in the starting phases of the cultures. The cells on the edges of the cultures dedifferentiated much sooner owing to local lack of matrix. The expression of collagen type X varied fairly considerably. Possibly, the expression of collagen type X in the 'young' cultures was correlated with the degree of osteoarthritis in the cartilage from which the chondrocytes had been isolated. This in vitro model appears to be suitable for the study of the regulation of the differentiation of chondrocytes and the effects of medications and biomaterials on the differentiation.

An animal model for the study of hematogenic infections of prostheses

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Infections of articular implants constitute a major problem and lead, apart from great suffering, to complicated and expensive revision surgery. The proportions of infection of total hip prostheses and total knee prostheses according to the literature amount to 1 and 4%, respectively. Little is known, however, about the pathogenesis of these infections, and especially of the socalled hematogenic infections. Consequently, the objective of the present study was to develop a reliable animal model for a hematogenically infected prosthesis/implant, which had to make evaluation possible of the methods of administration and doses of micro-organisms.

Material and method: Rods of titanium and of hydroxyapatite-coated titanium were implanted in the proximal right ribias of 10 NZW rabbits. After 4 to 6 weeks, the rods were contaminated selectively with different doses of Staphylococcus aureus (Wood 46). The contamination was brought about via a catheter introduced through the right carotid artery and advanced into the right femoral artery under fluoroscopic control. The evolution of the infection was assessed by determination of the body temperature, the BSE and the leukocyte count. One week after the contamination, the rabbits were sacrificed; one rabbit was only killed after 10 weeks. Culture material was collected from bone and marrow, spleen, liver, lungs and kidneys. The remaining bone with implant was processed for microscopy.

Results: After a dose of 10^9 cfu, the test animal died from sepsis. Contamination of a rabbit with a dose of 10^8 cfu was not followed by any clinical signs of infection. After one week, all cultures were negative. Another rabbit was given $3.3x10^8$ cfu. Since there were no signs of infection, this rabbit was not killed after 7 days, but continued to be followed clinically. No signs of infection developed even at a later stage. However, after being killed at 10 weeks, *S. aureus* was cultured from the bone along the prosthesis. The other animals were contaminated with $5x10^8$ cfu *S. aureus*. During the first few postoperative days, these animals showed slightly raised temperatures and slight increases of BSE and leukocyte count. After sacrifice, in most cases after one week, all organ cultures were negative for *S. aureus* with the exception of one culture of lung tissue. However, in all animals *S. aureus* could be cultured from bone and/or marrow beside the implant. The histological preparations showed incidental foci of mild infection. In some sections, bacteria were also visible in lacunae and small blood vessels. Two animals died, 2 and 3 days after contamination, as the consequence of an infection with Proteus mirabilis, for which no adequate explanation could be found. Proteus is a commensal in rabbits and a lowering of the resistance might lead to Proteus infection.

Conclusion: In this study, an animal model was developed by means of which hematogenic infections round implants can be studied. The model will be used subsequently to investigate the part played by the interface (the membrane between prosthesis and bone) in infections, and prevention or treatment of infections of this nature.

Practical and economic benefits of a hospital having its own bone bank

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Because of the increased demand for homologous bone tissue for transplantation purposes, femoral heads obtained during plastic surgery of the hip are often stored as donor bone. Minimizing the risk of transfer of diseases necessitates thorough screening and examination of the donor. This requires an adequate organization. By means of a review of 1.5 year's practice, an attempt was made to gain an impression of the practical and economic benefits of a hospital having its own bone bank.

Material and method: In Heerlen, in the period from 1 March 1992 to 1 September 1993, 330 operations with femoral head replacement were carried out. Of the femoral heads, 98 were stored with a view to donation. The bone bank procedure consisted of the following steps: selection of patient and bone by the surgeon, patient's consent, storage of bone (in a small sterile container) in a freezer and laboratory tests (bone culture and serological screening for HIV, syphilis, hepatitis B and C).

Results: Of the 98 femoral heads, 54 were ultimately transplanted. The donor bone was implanted in 49 patients; five patients received two femoral heads. Two of the 49 recipients postoperatively developed a wound infection. Of the 98 femoral heads selected for donation, 44 were ultimately rejected for transplantation (unsterile, unsuitable laboratory findings, medical contraindication, incomplete laboratory screening). Dividing the total expense of femoral head transplantation by the number of heads gave an amount of Dfl. 236,38 per femoral head. The market value of a comparable transplant at international bone banks ranges from Dfl. 700 to Dfl. 1.190.

Conclusion: In view of personal experience and on the recommendation of the Netherlands Orthopedic Association ('window periods' of virus diseases), the Heerlen bone bank protocol was adjusted (to include CMV-IgM). At present, serological screening is done only once, namely 6 months after removal of the bone, to avoid additional expense. The hospital's own bone bank has become indispensable. The bone grafts are far cheaper than those from international bone banks.

Bone transplantations in cemented knee prostheses

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Bone defects at primary or revision surgery of knee prostheses can be reconstructed with the aid of bone cement, metal inlays in modular systems, custom-made prostheses or bone transplantations.

Large defects should preferably be reconstructed using nonsolid bone grafts.

Material and method: Between 1989 and 1993, bone transplantations were carried out during implantation of a cemented total knee prosthesis in 36 knees of 30 patients. 45 bone defects were reconstructed, 31 of them in tibial and 24 in femoral localizations. 23 knees were subjected to a primary knee arthroplasty and 13 to a revision of a knee prosthesis.

Small defects (13) were treated with solid spongy grafts, while medium-sized (11) and large (21) defects were treated with impaction of spongy bone fragments in case of sufficient containment of the defect. In large femoral defects, in particular, solid corticospongy grafts (6) were necessary for lack of containment.

All prostheses were implanted with cementation; 34 times a non-constrained total condylar prosthesis (Kinematic, PFC), twice a constrained prosthesis (GSB, PFC).

Results: During a follow-up averaging 3 (1-5) years, two knee prostheses, in which at the time a femoral defect had been filled with large corticospongy grafts, developed subsidence with loosening. In 3 of the 14 femoral grafts, good assessment was made impossible by the metal of the prosthesis used. Eleven of the 14 large bone grafts in the tibia exhibited fine incorporation; only one tibial component showed demarcation without migration. The 17 other small and medium-sized tibial grafts had grown in well. The mean IKS score at follow-up was 90 (70-100).

Conclusion: Large tibial defects can as a rule be reconstructed with the aid of grafts in the form of impacted bone particles, with good clinical and radiographic results. Reconstruction of large femoral defects necessitates the use of solid grafts because of lack of containment; the risk of disintegration of the graft in the long run is then considerable.

Histological evaluation of reconstructions of bone defects with bone particle grafts

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Impacted bone particles have been used in our clinic since the late seventies for biological reconstruction of acetabular bone defects. Since then, a similar technique was developed for the repair of proximal femoral defects and currently, a method is being developed for the reconstruction of bone defects brought about by loosened knee prostheses. The clinical result of the reconstruction technique in the acetabulum is good compared with other reconstruction techniques. Still, with relatively new applications of the bone impaction technique, such as in the femur or in the knee, the need is felt of early evaluation of the behavior of the bone graft over time. Elements of particular importance are the rate and completeness of the remodelling of the graft to a new bone structure. Roentgen techniques as a rule are not suitable for the evaluation of the incorporation, because of the disturbing effects of the metal of the prosthesis or of the metal meshes used to reinforce the reconstruction. A detailed follow-up of the incorporation process is possible, on the other hand, by means of systematic histological evaluation of bone biopsy samples from the reconstruction. For this reason, the department tries to obtain as many biopsy samples as possible for histology. In order to obtain a complete picture of the incorporation of a graft, cooperation with other clinics using the same technique is indispensable.

Material: Needle biopsies were performed in a number of patients previously subjected to a bone transplantation when reoperation was necessary for some reason, without further impairing the reconstruction. Once this concerned an operation because of a persistent fistula after a femoral graft reconstruction (three months after revision), once an invasive repositioning of a dislocation (four months after grafting), while one patient was reoperated 11 months after a total hip prosthesis revision because of a patello-femoral problem. Larger tissue fragments from the interface between bone graft and cement were obtained at reoperation because of a persistent infection (one month after reconstruction) and, in another case, of a fracture distal to the stem of the prosthesis which brought about a pseudarthrosis (four months after reconstruction). Accordingly, biopsy samples were obtained 1, 3, 4 and 11 months after bone grafting reconstruction.

All biopsy samples were processed according to routine histological techniques.

Results: The biopsy samples with a host-graft interface showed good consolidation. The incorporation of the transplanted bone structure varied: after 3 to 4 months, the incorporation in a number of biopsy samples was almost complete and a new trabecular structure could be discerned. In the biopsy sample from the revised knee prosthesis, part of the graft was still avascular and not incorporated after 11 months. The cement penetration into the impacted bone was good. In a number of cases, contact was seen between newly formed bone and the cement mantle, although in most locations a fibrous separating layer was nevertheless present.

Conclusion: To obtain a complete picture of the rate at which a bone graft is incorporated, a number of biopsy samples are required. From the different histological images encountered in this study over time, we concluded that the rate of incorporation varies greatly. Possibly relevant factors are the location of the graft, the condition of the bone bed and the host and the mechanical stress on the graft.

Results of use of Polyactive™ in an animal model for surgical treatment of scoliosis

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A search for bone substitutes is in progress everywhere. Morbidity after removal of autologous iliac crest bone is considerable, mainly because of pain which is often protracted. Although a really good alternative to autologous bone will probably never be found, a number of biomaterials have meanwhile become available which (as a supplement to the osteoinductive properties of autologous bone) have good osteoconductive properties. Experiments with use of biomaterials have also been carried out in spondylodesis models with fusions over short tracts. The experiment reported here, unlike earlier studies, involved fusion over long tracts (5 or 7 levels), with comparison of the effects of autologous bone and those of an entirely new co-polymer, in granulate form, instead of the well-known co-polymer with a ratio 70/30 supplied in blocks as used successfully in other experiments for diaphyseal locations.

Material: In six goats, instrumentation and fusion were performed over a total of 28 levels, mostly in the lumbar tract. To begin with, the laminae were freshened and the facet articulations removed. Subsequently, autologous bone from the spinous processes and laminae was applied on the left side. On the right, use was made of Polyactive 80/20, a biodegradable co-polymer of polyethylene oxide and polybutylene terephthalate mixed with autologous bone in a proportion of 1:1, in granulate form.

In one animal, a fusion over five levels was carried out using the same spondylodesis material but no instrumentation.

Results: One test animal died after 4 weeks as the result of an infection with three different bacterial strains and a paraplegia, after a period without problems. In five test animals with instrumentation and one animal without instrumentation, the spondylodesis could be evaluated both macroscopically and microscopically after 3 or 6 months. Macroscopically, there was always a distinct difference between the side of the spinal column with exclusively autologous bone and the side with the mixture of autologous bone and Polyactive. Use of the autologous material virtually always resulted in a firm spondylodesis, apart from a few pseudarthroses, while the mixture of bone tissue and polyactive material after 6 months always showed a crumbly and more fibrotic appearance. At microscopy, this difference was also observed histologically: a fine bone bridge on the side of the autologous bone, a thinner bone mass over the laminae on the side with the bone/Polyactive mixture where after 3 months the developing bone mass was still found to contain remnants of Polyactive. After 6 months, these remnants could no longer be discerned clearly.

Conclusion: Use of this new co-polymer in the proportion 80/20 in granulate form, mixed with autologous bone, resulted in inferior bone formation compared with exclusively autologous bone or the co-polymer with the 70/30 proportion in block form as described elsewhere. An explanation of the difference in result is possibly to be found in the fact that Polyactive 80/20 swells much more than the original material in block form and that moreover the graft in the back was subject to relatively little stress and was in contact with bone tissue only on the side with the freshened laminae.

Histological quantitative analysis of ingrowth of bone into the biomaterial Polyactive™ in various localizations

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A study in laboratory animals was carried out in order to assess the quality of newly formed bone in the biomaterial Polyactive™ (PA) and the degradation of this biomaterial. The amounts of bone in cylinders of PA were measured in the cortical bone, in the marrow and in the spongy bone of rabbits. Biomaterials currently available all to a greater or lesser extent contain calcium phosphates such as hydroxyapatite, tricalcium phosphate and glass ceramics. These biomaterials are considerably more rigid than bone, and most of them are also relatively brittle. Implantation of these materials into spongy bone results in a compact calcified mass. Previous investigations have shown that the biomaterial PA with its elastic qualities led to ingrowth of bone without the necessity of osteoinductive additives.

Material and method: The amounts of newly formed bone in a recently developed resorbable co-polymer of 60% polyethylene oxide and 40% polybutylene terephthalate were studied in cortical and spongy bone. Use was made of 128 rabbits at least 6 months old. A cylinder of PA with communicating pores was introduced into a hole drilled into the cortex of the femur just distally to the trochanteric region, 4 mm in diameter and 5 mm in depth. An identical cylinder was implanted in the spongy trochlea of the knee. Sacrifice and quantitative histological analysis (image analysis, Quantimet, Leitz, Germany) were done 4, 8, 26 and 52 weeks after implantation of the cylinders.

Results: A rapid increase of the proportion of pores filled with bone was seen in the femoral cortical bone between 4 and 52 weeks; as early as 8 weeks after implantation, 91% had filled with bone. Degradation of PA was seen to begin after 8 weeks. The degree of degradation of the biomaterial was difficult to measure because of its swelling capacity. The findings included not only cell-mediated degradation from the outside (macrophages) but also hydrolysis within the biomaterial itself.

Owing to this degradation, the proportion of bone-filled pores had decreased to 83% after 52 weeks. The numbers of

giant cells per defect were small. In the marrow, the pores filled with bone substantially less than in cortical bone: after 4 weeks, 10% of the pores were filled with bone, after 8 weeks 2.6% and after 26 weeks and 52 weeks, the proportion was zero. In the spongy bone, also, much less bone formation was seen than in the cortex, the proportions of bone in the PA ranged from 14% to 28% after 4, 8, 26 and 52 weeks.

Conclusion: Bone formation in the PA cylinders was rapid, especially if they had been implanted in cortical bone. In contradistinction to other biomaterials which require bone-binding additives, the degradation of PA in this study was always followed by bone formation on which degradation products did not exert any adverse influence. PA has specific bioinductive qualities, unlike biomaterials such as hydroxyapatite and tricalcium phosphate. After the initial calcification and bone formation in the medullary canal, bone formation stopped, while bone already formed was resorbed and replaced by normal bone marrow and connective tissue.

The influence of hydroxylapatite coating on the incorporation of an allograft

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The incidence of revision operations of articular prostheses is bound to increase in the future, in spite of improved operation and cementation techniques. The results of an arthroplasty as a rule deteriorate with time, due to mechanical loosening. A main problem at revision operations is the bone defect that remains after removal of prosthesis and cement. If transplantation is carried out in such a situation, its purpose is to supply osteogenic cells, osteoactive factors, an osteoconductive matrix and structural integrity. An allograft supplies osteoconductive properties and sufficient structure for the later bone production. Reports in the literature concerning the use of allografts at revision operations vary. Little is known, however, about the additional influence of use of hydroxylapatite at bone grafting. An experimental study was carried out in order to determine whether an implant coated with hydroxylapatite gives better incorporation of the allograft than an uncoated graft.

Material and methods: Titanium implants with grooves on both sides were implanted into the two distal femora of Alsation dogs. Two types of implants were used: one with a groove measuring 15x5x3 mm and the other with a groove measuring 15x2 mm on the surface and 2.3 and 5 mm in depth. One side of the implant was coated with hydroxylapatite with a crystallinity of 64% and a thickness of 50 micron. The other part was left uncoated. After preparation of the femur, the grooves were filled with spongy allografts. Three of the total of six dogs were sacrificed after 4 weeks, the other three after 8 weeks. Eight and 2 days, respectively, before the sacrifice of the animals tetracycline was administered to label newly formed bone.

Results: Ingrowth of bone into the grooves was seen on both sides of the implants, but it was most pronounced on the side coated with hydroxylapatite. This was confirmed by the tetracycline labelling. After 4 weeks, osteointegration and incorporation were to be observed in the bone grafts near the hydroxylapatite coating. The uncoated sides showed highly variable results.

Conclusion: Hydroxylapatite coating of an implant exerts a positive influence on the ingrowth of bone and the incorporation of a bone graft.

Use of hydroxylapatite to fill bone defects after excochleation of benign bone tumors—clinical and radiographic results in 15 patients

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Hydroxylapatite is a naturally occurring calcium phosphate compound that can be used to fill bone defects after loss of bone due to injury or operations, e.g. after excochleation of benign bone tumors. It is especially if a large amount of spongy bone is required and/or the amount available is small, that hydroxylapatite can offer a solution.

Material and method: 15 patients with a mean age of 24 (7–48) years were followed up postoperatively for an average of 41 (1–101) months. A histological diagnosis had been made in 12 cases: giant cell tumor (4), aneurysmal bone cyst (3), juvenile bone cyst (3), fibrous dysplasia (1), chondroblastoma (1). The treatment comprised excochleation of the lesion, combined in nine cases with cryosurgery, followed by filling of the defect using autologous spongy bone and hydroxylapatite in granulate form.

Results: The radiographs in all 15 cases indicated a completely obliterated bone defect with unchanged presence of hydroxylapatite. In the course of the follow-up, tumor growth recurred in the youngest three patients (7, 9 and 14 years), two of them with an aneurysmal bone cyst and one with a giant cell tumor. Once more the chosen treatment was excochleation in combination with cryosurgery and filling with autologous spongy bone and hydroxylapatite. At the last follow-up examinations 13 patients were free of symptoms; they had normal function of the extremity involved.

Conclusion: Hydroxylapatite can be used to fill bone defects resulting from excochleation of benign bone lesions. It is especially in cases where much spongy bone is required or the available amount is small, as in young children, that this biomaterial offers a good solution.

Analysis of biodegradable osteosynthesis material in laboratory animals and in patients

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Biodegradable osteosynthesis material does not have to be removed surgically after healing of the fracture. The material must remain strong for long enough to allow consolidation of the fracture. In addition, the bioresorption process must have no adverse effect on the bone healing. Clinical results have to be similar to those obtained with metal plates and screws. Biodegradable rods (diameter 4.5 mm) made of polyglycolic acid (PGA) coated with polydioxanone (PDS) were investigated in vitro, in animals and patients.

Material and method: The biodegradable material was produced by compressing PGA suture material at rising temperatures (215–232 °C) and pressure (80 N/mm^2) and coating it with a layer (0.5 mm) of PDS (5% w/v). To begin with, the change of pH in vitro was measured during 10 weeks at 37 °C in physiological saline and in two different buffer solutions (Gomoris' trismaleate and Sørensen's reagent) with and without PGA-PDS rods (length 10 mm, diameter 4.5 mm, weight 300 mg). In-vivo studies were carried out in six dogs (beagles, males, 15 kg). On both sides, the femoral shaft was reamed out lengthwise (100 mm, diameter 4.5 mm), transverse holes were drilled in the proximal tibia (diameter 4.5 mm). On the right side, the PGA-PDS rods were implanted into the femur (diameter 4.5 mm, 70 mm) and into the tibia (diameter 4.5 mm, over the entire width of the plateau). The left side was used for control purposes. Follow-up examinations were carried out after 6, 10 and 16 weeks with radiography, technetium scintigraphy, histology and mechanical testing. To conclude, a series of 34 patients with ankle fractures, with over 2 mm dislocation of the lateral and/or medial malleolus, were divided at random into two groups. Group 1 was treated with PGA-PDS rods, group 2 with plates and screws according to the AO method. Follow-up, with clinical and radiographic examination, was done after 3, 6 and 12 months.

Results: In vitro: in 10 weeks, the pH of physiological saline decreased from 7.0 to 2.5, that of Gomoris' trismaleate from 7.1 to 5.1 and that of Sørensen's reagent from 7.2 to 6.1. The pH values of the controls changed from 7.0 to 5.0 and from 7.2 to 7.3, respectively. The differences were statistically significant.

In vivo: Radiographs after 16 weeks showed no bone formation in the tibial drill hole with implant (radiolucent material) while the control drill hole had filled with bone tissue. Histological preparations showed no lymph node activity, there was a fibrous layer surrounding the implant and the implanted material showed degradation after 16 weeks. The changes in strength between the implanted and the non-implanted sides 6, 10 and 16 weeks after the operation in the femur amounted to 27%, 74% and 0%, respectively. In the tibiae, these proportions were 48, 26 and 14%, respestively. The ratios between the right and left femora in activity storage at technetium scintigraphy after 3, 6 and 16 weeks amounted to 15%, 47% and 4%, respectively. In the tibias, these proportions were 73%, 15% and 11%. The term of the maximal mechanical strength of the material postoperatively was found to be 10 weeks.

The operated on patients developed no complications, more in particular no dislocations exceeding 1 mm. There were no differences in results between the experimental group and the control group (Olerud scores 96 and 92 points, respectively). No sinus development was seen in the radiographs of the ankles.

Conclusion: The degradation of the study material in vitro was accompanied by a significant fall of the pH. It appears that in vivo this phenomenon does not affect the fracture healing in the long run, but this question deserves further investigation. Technetium scintigraphy appears to be a possible parameter for the process of degradation of the PGA-PDS rods. In this study, sufficient stabilization of ankle fractures could be achieved with PGA-PDS, with results similar to that of surgical treatment according to the AO principles.