JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 345–351

Hydroxyapatite-ceramic for juxta-articular implantation*

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The histocompatibility of hydroxyapatite-ceramic (HAC) has been proven extensively. For the reconstruction of juxta-articular cancellous bone defects with this synthetic material, the mechanical properties of the HAC–bone regeneration complex needed to be investigated. In order not to alter the specific ability of the articular structures to distribute and absorb loading stress, the physiological force-transmitting performance of the subchondral zone must be achieved by filling the defect within HAC. This study deals with the influence of a physiological load on the remodelling within HAC-filled subchondral bone defects. As orientation is the important factor affecting the physical properties of hard tissue, we show the morphological aspect of functional adaptation of the hydroxyapatite–bone compound determined by the orientation of the bone collagen fibres. By biomechanical methods, the elastic properties of the resulting ceramo-osseous regeneration complex were tested. Reproducible subchondral bone defects were prepared in medial femoral condyles of rabbits, leaving a 0.5 mm coplanar layer of bone and cartilage. The defects were filled with granules of HAC. Polarizing microscopy revealed the dynamical aspect of the bony integration of the material and the remodelling process under physiological locomotion. It showed a rapid ongrowth of collagen fibres on the ceramic surface. By its increasing orientation to domains from woven texture to economical trabecular architecture, the load-bearing facility is documented. Indenting the articular surface on an impressive force testing machine 18 months after HAC implantation proved the equal elastic response of the ceramo-osseous regeneration complex with the overlying structures in comparison with the integrity of not-operated femoral condyles. When integrated by bone, HAC fulfils in our dynamic animal model physiological demands even in large bone defects close to articular surfaces.

1. Introduction

Articular structures distribute and absorb physiological stress by deformation. Subchondral lamellar bone and the epiphyseal trabecular arrangement work as a cushion shielding the overlaying cartilage from lesions by peak forces [1]. Alterations in the quality of the subchondral bone could have a profound effect on the ability of a subchondral bone–articular cartilage system to withstand compressive dynamic forces [2].

Juxta-articular cancellous bone defects resulting from cysts, trauma, infection and tumour resection need to be filled to avoid joint damage. Autologous cancellous bone is still the most widely used material for filling bone defects. Ample defect zones require massive transplants, which often exceed the patient's bone resources. Using synthetic substitutes, the success of such an operation depends on the functional compatibility of the material used for this purpose.

The histocompatibility of sintered hydroxyapatite-ceramic (HAC) has been proven extensively with cell culture and implantation experiments [3–7]. Chemical and physical tests [8–12] have revealed its structural similarity to natural bone mineral, which makes its conductiveness to the ingrowth of bone one of its most impressive properties. Various authors have characterized this as "osteotropic" [10] or "osteophilic" [11]. Even stimulation of certain osteogenic factors has been claimed [13]. A principal limitation of native HAC is that it is brittle and performs with less tensile and bending strength than bone. Studies with implantation under physiological stress in the tibial shaft [14, 15], femur [13] and jaw [9] have dealt with this problem. Implantation of HAC close to joint surfaces with possible influence on the integrity of the articular structures has not yet been studied, regardless of clinical practice, but defects in that particular

* On the occasion of his 60th birthday, we dedicate this study to Professor K. H. Jungbluth, Head of Trauma and Reconstructive Surgery Department, University Hospital of Hamburg.

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localization have the highest demand of filling material for reconstructive surgery of bone.

Calcium phosphate bioceramics as bone substitutes or extenders can be used for weight-bearing implantation only when reinforced by bone ingrowth [16]. As no significant degradation of bony integrated pure HAC has been demonstrated in previous studies [10, 16], a permanent influence on the mechanical properties of the defect filling bone by the integrated HAC has to be expected. Alterations in the subchondral elasticity after implantation of HAC would lead to degenerative joint disease [17]. It is reasonable to ask whether HAC alters the performance of the sensitive articular structures after juxta-articular substantial integration under physiological loading. Therefore, the mechanical adaptation of the resultant tissue–implant composite system is of special interest for the final result of its application as a synthetic bone alternative.

We used a dynamic animal model to investigate the developing ceramo-osseous regeneration complex. By implanting the synthetic material close to articular surfaces, the bone remodelling with the HAC is controlled and influenced by stress during locomotion.

With polarizing microscopy we studied the morphology of the functional adaptation of the HAC–bone compound as a function of the orientation of the bone collagen fibres. In addition, the articular structures in this experimental design may be used to test the stiffness of the underlying trabecular system.

We have previously [18] reported a study that involved the same bone preparation procedure used for this present study. Instead of filling the defects with HAC, they were left unfilled or were filled with frozen homologous cancellous bone grafts. At latest 12 weeks after the operation, all articular surfaces over the filled or unfilled defect areas were destroyed by collapse of the subchondral compartment with fractures and resorption of the subchondral bone plate. Thus, the defect filled with fibrous connective tissue, fibrocartilage replacing the destroyed hyaline articular surface.

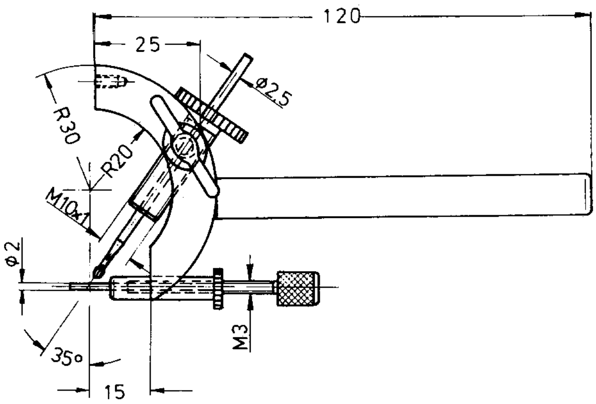

Figure 1 Design for the "stereotactive" guiding device for the dental burr to perform reproducible bone defects leaving a coplanar hot layer of 0.5 mm cartilage and subchondral bone plate. Not shown is the pneumatic mini-drill-turbine. Dimensions in mm.

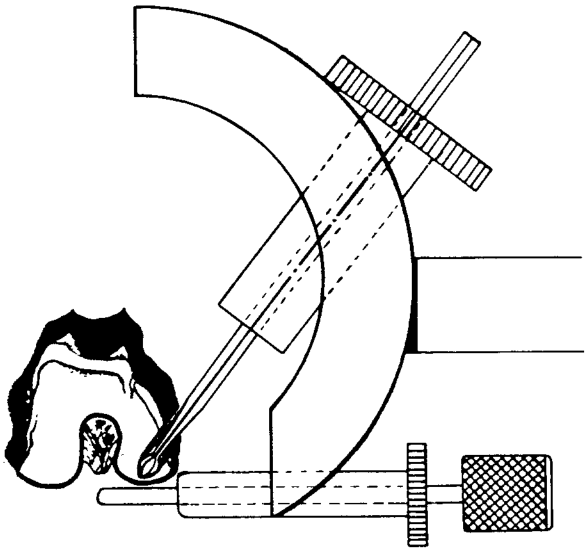

Figure 2 Schematic diagram of the dental burr in the drilling position guided by the especially designed holder. For close contact of the limiting brass pin to the articular surface of the femoral condyle it must be inserted through an incision into the flexed knee joint capsule distally of the medial meniscus.

2. Operative procedure

In adult "Deutsche Riesen" (German giants) rabbits of 3.9 $\pm$ 0.2 kg body weight we drilled large reproducible subchondral bone defects 3.1 mm in diameter below the articular surface of the medial condyles. Under general endovenous anaesthesia with ketamin hydrochloride, the medial metaphyseal plane of the distal femur was exposed by a longitudinal incision placed anteromedially. The anterior part of the medial compartment of the knee joints was opened by incision of the joint capsule and synovium distally of the anterior horn of meniscus. A dental burr was inserted from a cortical portal ventral to the medial collateral ligament insertion and guided by an especially designed stereotactic device, which rested with its limiting pin on the articular surface of the condyle (Figs 1 and 2). The drilling left a 0.5 mm coplanar layer of subchondral bone and cartilage at the central weight-bearing area for the common squatting positions of rabbit hindlegs (Fig. 3).

The canal of the drilling was thoroughly irrigated and bone fragments were washed out. The defect was filled under slight pressure with granules of porous HAC, previously soaked in blood for 5 min. Bilateral incapacitation was gained by simultaneous operation of both femurs.

For mechanical testing controls we used five non-operated joints. Defects left unfilled could not be used for indentation experiments, as we have shown that these joints undergo massive degenerative changes after collapse of the articular surface [7].

All rabbits were allowed to move freely in their large cage (3600 cm^2) after the operation. No additional exercise was performed. The rabbits were fed Altromin standard diet and water *ad libitum*.

3. Materials

In this study we used HAC granules of Osprovit (Cerasiv Ceramic Division, Plochingen FRG). Pure

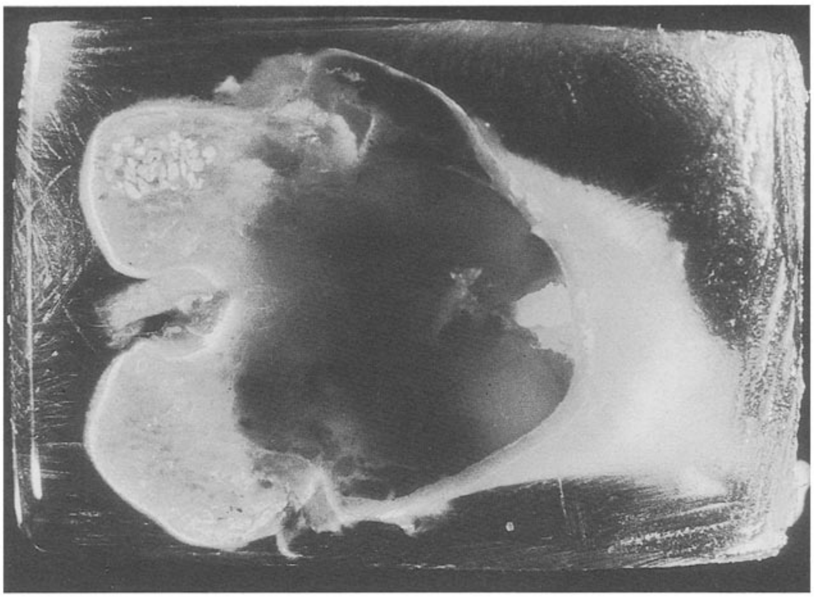

Figure 3 Coronar section plane through the (left) femoral condyles with clearly visible integrated ceramic granules of hydroxyapatite below the articular cartilage and subchondral bone plate. Macrophotography of the resin block in the position for histological preparation.

synthetic pentacalcium monohydroxyorthophosphate ($Ca_5(PO_4)_3OH$) powder was sintered with the addition of H_2O_2 to give a stoichiometric ceramic material with interconnecting porosity of 42% and macropore size of 100–400 μm. The particle size used for this purpose was ≤ 0.8 mm. The Vickers hardness was 500 MN m^{-2}. This material was produced according to ASTM F 1185-88 regulations (Standard Specification for Composition of Ceramic Hydroxyapatite for Surgical Implants), which characterize the physical and chemical properties of sintered calcium phosphate ceramics.

4. Evaluation methods

For the polarizing microscopy study the rabbits were killed 2, 12, 24 and 36 weeks after the operation. The femurs were removed and the soft tissue dissected from them. The undecalcified fixed specimens were embedded in methyl methacrylate and sectioned in a plane parallel to the defect canal (Fig. 3) by a sawing-and-grinding technique [19]. No staining was applied for evaluation between crossed nicol prisms. The quality of the primary 60–100 μm slices depended on the rigid fixation of the specimen block and the robust precision parallel control of the bandsaw unit. A cooling and flushing system prevented unwanted heating of the sample. The grinding of the slide to a specimen thickness of 10–5 μm was done in three steps by sandpaper on a grinding machine. A Zeiss polarization microscope was used for examination and photodocumentation.

At the end of the experimental period, 18 months after the implantation, 10 rabbits were killed by intravenous injection of pentobarbital. We immediately removed the distal femora from the rabbits. Macroscopical examination of the articular surface of each specimen was documented with colour photographs. Three steel claws secured each femur (freed of surrounding soft tissue) to a solid steel plate mounted with a ball-and-socket joint on the base of a hand-driven mechanical testing machine (Reicherter EE2000). In various positions the distal femurs underwent impressive force tests up to 50 N by a plane-ended indentor 1 mm in diameter in perpendicular contact on the articular surface, which was permanently irrigated with Ringer's solution (Figs 4 and 5). The indentor diameter was selected to be smaller than the defect area in order to allow central indentation fully influenced by the subchondral stiffness. The indentation was performed at a crosshead speed of 0.6 mm min^{-1}. In order not to destroy the integrity of the distal femur, we indented within the limits of the physiological load zone. In a second test series the cartilage was carefully resected and the measuring process was repeated on the subchondral cortical bone plate.

We demonstrated reproducibility by repeating the indentation in the same position after 5 min and a difference of less than 5% was documented in the displacement values in comparison with the first test series.

For biomechanical testing, 18 reproducible indenting points had to be defined, six positions at equiangular intervals (15 °) over the condylar surface on each of three equidistant arcs as shown in Figs 6 and 7. The indentation depth was measured by a mechanical displacement register in steps of 0.01 mm. Its tip was placed on the upper end of the indentor assembly. We plotted the stress–deformation rate continuously by feeding the signal from the testing machine into an *xy*-plotter. The curves underwent linear regression from 10 to 50 N.

Preliminary investigations [7] have proved the sensitivity of our experimental design: leaving the subchondral cancellous bone defects empty and keeping the rabbits on normal locomotory activity is followed by complete depression of the articular surface within 8–12 weeks. The same destructive effect could be verified after implantation of frozen homografts.

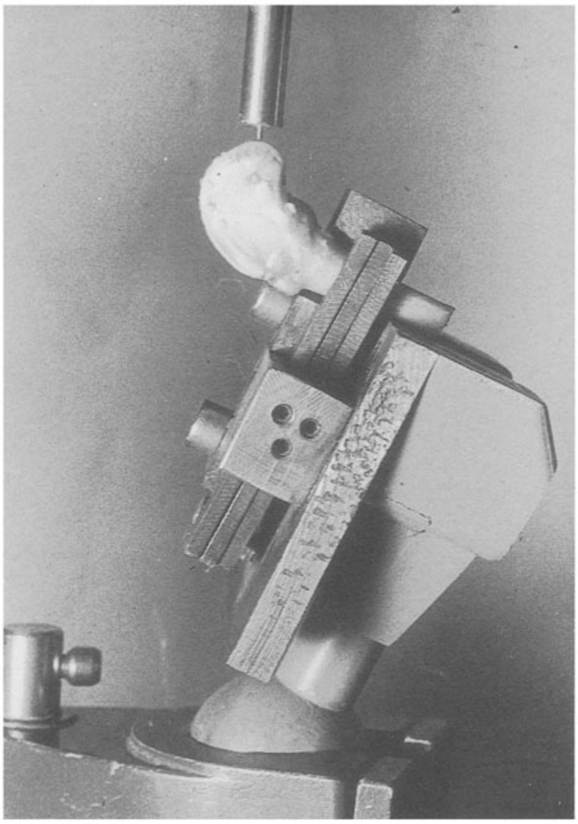

Figure 4 Distal femur part in the measuring position, secured with claws to a solid ball-and-socket joint on the platform of the compressive testing machine. The steel indentor is in perpendicular contact on the articular surface.

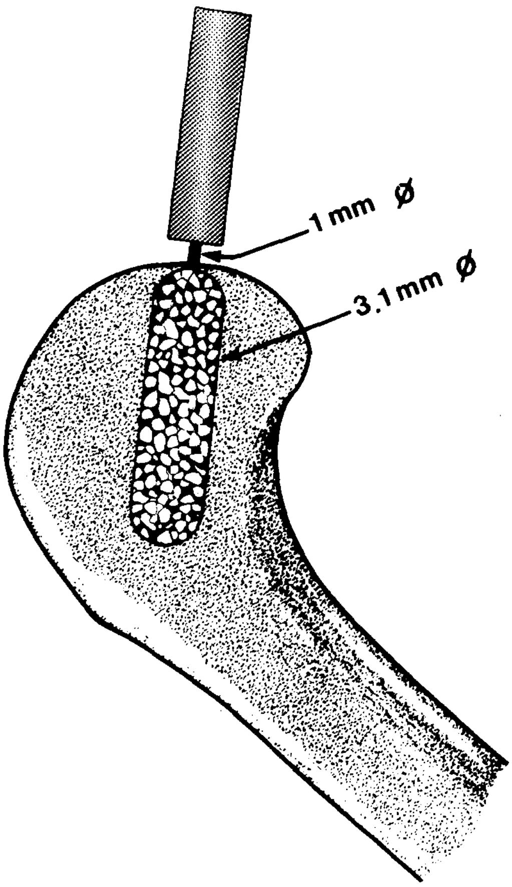

Figure 5 Lateral view showing the relationship between the defect zone with the implanted complex of HAC granules below the 0.5 mm coplanar layer of subchondral bone and cartilage and the indentor position in perpendicular contact on the articular surface used for compressive force testing.

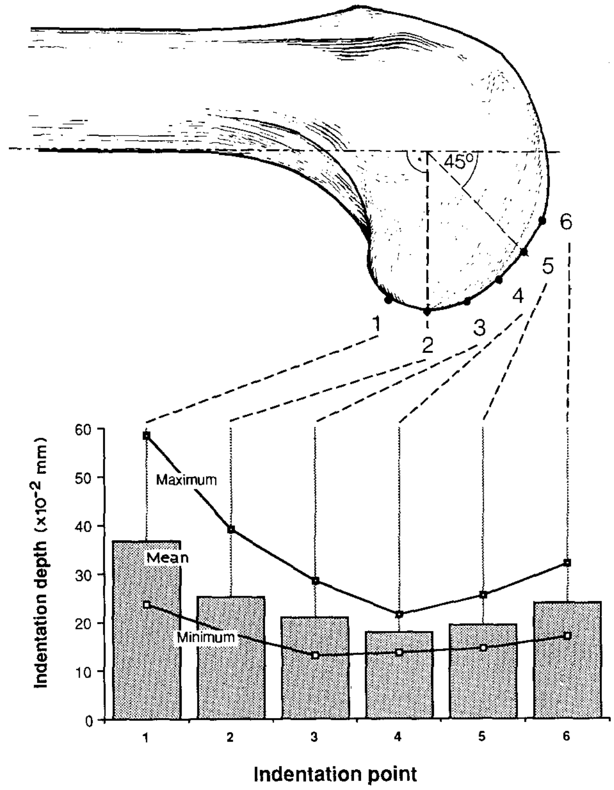

Figure 6 Lateral view showing the distribution and numbering system of the indented areas on the medial femoral condyle in its relationship to the columns of the mean indentation values. Overlay chart for minima and maxima.

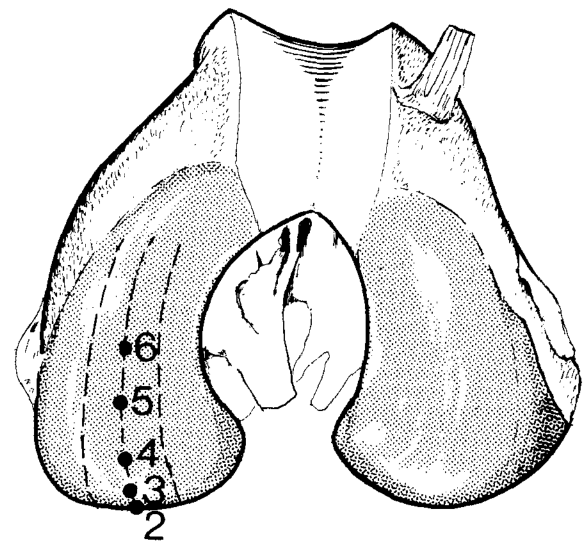

Figure 7 Anterior view of the left femoral condyles of a rabbit's knee joint with three equidistant lines; on each the indentation positions are numbered 1–6, as shown here for the centreline.

Over the same period, for defects with cancellous autografts, harvested from proximal tibia of both sides, no morphological changes in the integrity of the femoral condyles were noticed. By intensive osteoblastic and early remodelling activities, a trabecular framework developed with full functional adaptation.

5. Polarizing microscopy results

This study of the integration process revealed by polarizing microscopy, two weeks after implantation of

HAC granules, the formation of a web of collagen fibres with low content of distinctly oriented material. Spreading over the entire ceramic surface and the walls of the defect we saw a simple and short trabecular network of bone fibres. No limiting tissue encapsulation on the HAC granules inhibited the direct ongrowth of collagen matrix (Fig. 8). The predominant optical axis of the fibres did not deviate from the granules. Bone collagen fibres encased closely the ceramic implant and originated from the surface of the granules. Until the end of the 12th week the filling of spaces between all ceramic granules and the borders of the defect occurred with densely packed and highly orientated fibres of bone. The fibrillar architecture of cartilage and subchondral plate visible with polarized light did not undergo any change during the entire course of the study.

By 24 weeks the remodelling process yielded a still-increasing orientation and fully organized lamellar bone texture between the ceramic granules. The bony component of the restorative mass was gradually reduced to an economical and optimized structure. This was visible with no significant alterations in all parts of the defect until the end of the histological examination 36 weeks after the operation. Newly developing bone-marrow areas gave back the spongy structure to the former defects (Fig. 9). However, integration of the ceramic was not precisely uniform. There was a longitudinal predominance of trabeculae with narrow connections, as was also found in native femoral condyles.

During the course of the study there was no obvious alteration in the spatial arrangement of the trabeculae (trabecular contiguity ratio) [19] outside the defect area. This means that the number and the diameter of the laterally continuing trabeculae remained unchanged. The specimens provided too few trabeculae for statistical analysis. There was no evidence of ceramic fragments from a possible degradation process outside the defect canal by typical crystallites double-refraction with polarized light.

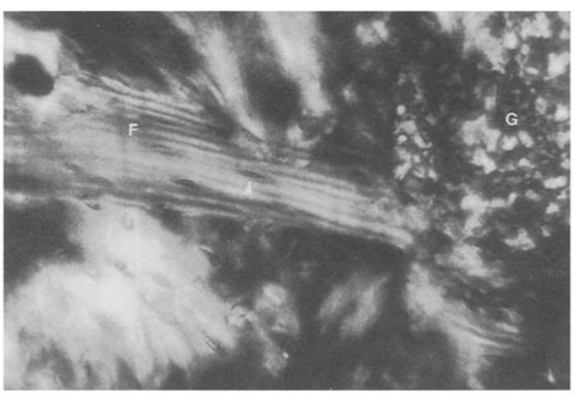

Figure 8 Polarizing microscopy reveals by light reflection through crossed nicol prisms the orientation of woven bone collagen fibres (F) and their direct ongrowth on the surface of the "osteotropic" ceramic material. Double refracting the crystallites of the hydroxy-apatite granulum (G). Bone cells displaying as empty spaces (arrow).

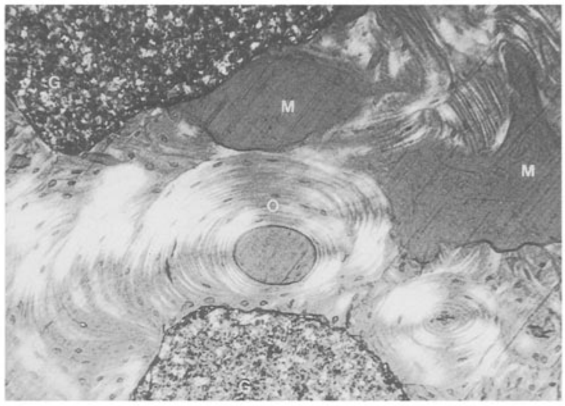

Figure 9 Polarization micrograph of the fibre orientation of fully organized osteonal bone filling the room between ceramic granules. Such is the result of remodelling under the permanent influence of physiological locomotion, transmitting force through the bone–ceramic regeneration complex. Newly formed bone-marrow cavities are an essential mark for economical bone architecture. G, ceramic granules; M, bone marrow space, and O, osteon.

6. Biomechanical findings

The morphological views of the bony regeneration complex with the substantially integrated ceramic material have biomechanical correlations. The femurs harvested 18 months after the implantation of the ceramic material did not show any damage of the articular surface macroscopically. The cartilage was smooth and shiny. The subchondral bone below it was completely intact. All indentations demonstrated reversibility when the load had been removed. No articular fracture happened during the mechanical testing. We evaluated eight distal femur portions with integrated HAC and five non-operated portions. A total of 468 indentations were carried out and correlated. The indentation values at 50 N impressive force ranged from 0.1 to 0.58 mm on the intact joint surface and from 0.06 to 0.28 mm after removal of the cartilage. There was no difference between the results for the right and left femurs of the same rabbit, but small inter-individual differences in the indentation values for corresponding areas of different rabbits. The mean displacement values for the defined measuring points were graphed and overlayed with the minimum and maximum.

With marked consistency from these descriptive statistics (Fig. 10) we found the following. All curves harmonized in shape, with similar topographical variation. The indentation had its greatest values at areas 1 and 6. The deformation–stress relation was lowest between areas 3 and 4. The indentation of the cartilage-free subchondral bone resulted in less absolute elastic deformation, but with the same variation over the entire testing field of 18 areas (rows 3 and 4). Finally, there was no discrepancy in the curves of mean indentation over HAC-filled defect areas (rows 1 and 3) in comparison with non-operated rabbits (rows 2 and 4).

7. Discussion

Surgical defects of dimensions such as those drilled for this study do not heal spontaneously without filling, as

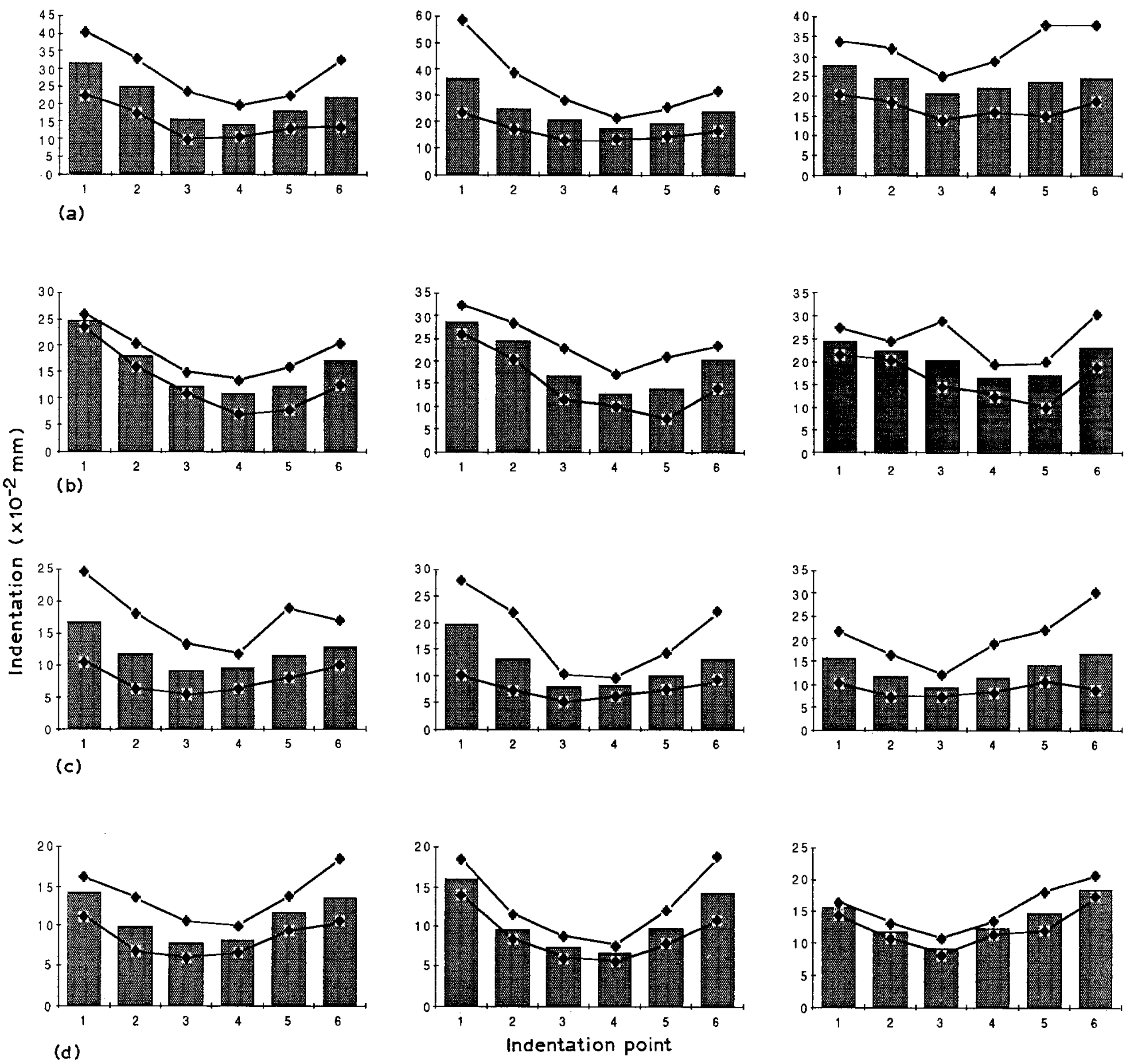

Figure 10 Graphs showing columns 1–6 of the mean indentation values versus observation points. Three distinct arcs, on which the six indentations were performed, are defined as medial (left column) central (middle columns) and lateral (right column), regarding their position on the medial condyle. Overlay line chart for minima and maxima. (a, b) Indentation tests on the articular surface and (c, d) after removal of the cartilage; (a, c) with and (b, d) without HAC.

our early work confirmed [18]. In the absence of regenerative bone the lack of mechanical counterbalancing of the cancellous framework is the reason for early 100% collapse of the articular structures. The ability of granules of HAC as a bone substitute to satisfy the mechanical demands of the normal use of the leg during the early phase of the integrational process is concluded from the intact articular surface at any time of the study.

With the polarizing microscope it is possible to demonstrate the filling of the peri-implant space with woven bone of low orientation index. The ceramic granules come under the influence of physiological loading by direct ongrowth of the collagen fibres on the surface architecture. This initial bony stabilization of HAC granules in the manner of "bonding osteogenesis" [10] is essential for the mechanical integration of this largely non-resorbable material and the functional performance of the bone–implant complex.

Later, according to Wolff's law the architecture within the defect zone is changed by adaptive remodelling [20] of the peri-implant bone as dynamic forces are applied across the implant. A spongy framework is restored by the development of bone-marrow spaces. On the other hand, there is no increase or decrease in cancellous bone mass and contiguity [19] outside the defect area as an inevitable consequence of any deviation of forces.

The ceramic material is far more than a simple "space filler" [9] when used as a bone substitute in implantation sites under physiological loading. For the HAC that we used for this study we did not find the limitation of the usefulness of calcium phosphate ceramics to areas without mechanical requirements, which is stated as a common drawback for materials with interconnecting porosity [21].

For possible stiffening of the subchondral area by the implantation of the ceramic we never found any indicator: there were no alterations in the fibrillar texture of subchondral bone or cartillage as fibrillation, or signs of cloning over a defect area, and no reduced indentation values on the articular surface. Moreover, the documented unaltered deformation–stress relationship is a valid criterion for separating normal joint surfaces from osteoarthritic ones [120].

Indentation tests on articular surfaces were applied as early as 1926 for the determination of cartilage elasticity [124]. These *in vitro* techniques have been improved since then. For femoral head indentation a similar measuring point definition comparable with our identification and numbering system were used [123]. Creep modulus determination for articular cartilage *in situ* was the aim of this study on the joint surface of entire proximal femurs, as indentation of cartilage was influenced by the proximity of the subchondral bone. However, the relatively stiff cancellous bone is capable of making a contribution equal to that of the thin articular cartilage layer in the attenuation of dynamic peak forces [2].

8. Conclusions

The aim of this study was not to determine the absolute value of the elastic modulus of the HAC–cancellous bone regenerate complex, but rather its relative performance against native subchondral cancellous bone within the integrity of the femoral condyle. The evaluation of the force–deformation curves revealed that the elastic properties of the whole joint load-bearing area, consisting of articular cartilage, subchondral bone plate and epiphyseal cancellous bone with the ceramic implants showed no significant differences in the topographical survey in comparison with untreated rabbits.

In our dynamic experimental design functional adaptation for mechanical demands led to a trabecular architecture with substantially integrated ceramic material. This compound system performs physiological force transmission even when used to bridge large juxta-articular bone defects. The histocompatibility and "osteotropic" bone-binding facility is paired with the functional compatibility of HAC as an integral part of living bone.

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Received 14 February
and accepted 11 November 1991