



Mechanism of bone incorporation of β -TCP bone substitute in open wedge tibial osteotomy in patients

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Abstract

A histological study was performed of bone biopsies from 16 patients (17 biopsies) treated with open wedge high tibial osteotomies for medial knee osteoarthritis. The open wedge osteotomies were filled with a wedge of osteoconductive beta tricalcium phosphate (β -TCP) ceramic bone replacement. At the time of removal of the fixation material, core biopsies of the area where the β -TCP was located were taken at different follow-up periods (6–25 months). β -TCP resorption, bone ingrowth and bone remodelling were studied. We hypothesized that the incorporation and remodelling process occurs similarly as in animals.

Histology showed a good resorption of the β -TCP with complete incorporation and remodelling into new bone. The different phases as described in animal studies were found. A correlation was found between histological findings and radiological assessment.

In conclusion, β -TCP appeared to be a bone replacement material with optimal biocompatibility, resorption characteristics and bone conduction properties for the clinical use.

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1. Introduction

A valgus high tibial osteotomy (HTO) is the treatment of choice for medial knee osteoarthritis and may be performed with a lateral closing wedge or a medial open wedge technique [1–10]. In case of a medial open wedge osteotomy, the bone gap created during the osteotomy can be left open, or filled with autograft or allografts. Furthermore, nonresorbable or resorbable bone substitutes can be used [1,11–15].

Autograft bone is generally considered to be the most successful bone filling material because of its osteocon-

ductive, osteoinductive and osteogenic properties [16–19]. Drawbacks of autograft use are increase of operation time and co-morbidity following the bone harvesting at the iliac crest. Allografts entail the risk of virus transfer or may be less potent to stimulate new bone formation. To encounter these problems, synthetic bone substitutes have become more popular in the last 20 years [16,18,20]. Because of their synthetic origin, these materials cannot cause disease transmission. It has been suggested that bone substitutes can accelerate bone growth and bone remodelling which may lead to earlier full weight-bearing after open wedge HTO [11,17–20].

Particularly porous beta tricalcium phosphate (β -TCP) is a very promising osteoconductive, ceramic bone substitute. β -TCP has slightly different ratios of

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calcium and phosphate than bone, but, in essence, the material properties are much like the inorganic phase of bone, which constitutes 60–70% of human bone [18,20]. The macro-porosity of the material facilitates bone ingrowth. The resorption characteristics depend on the mineral composition and the degree of sintering as well as the porous structure [18,20–22]. In animal studies tricalcium phosphates have shown favourable biocompatibility, osteoconduction and resorption properties. It appeared that the β -TCP gradually resorbs and in the end is completely replaced by remodelled bone [11,16–21,23–28].

The resorption of β -TCP and its conversion into bone takes place in different phases. In the first phase, there is some resorption of host bone, followed by apposition of new bone on the TCP scaffold. During this phase the appearance on X-rays is that of dense bone. In the following phase, there is further resorption of the β -TCP and remodelling of the bone and the density on X-rays decreases [11,17].

In patients, the incorporation was until recently assessed with standard radiographic examination. However, it is not easy to classify bone healing and distinct between bone and the remaining synthetic calcium phosphates on radiographs alone [16]. Based on an expected similarity of X-rays of patients with that of animals, a more refined radiological classification system was proposed to monitor bone healing in open wedge HTO with resorbable substitutes [11]. In this classification system (see Section 2 for details), five distinct phases can be discriminated. So far it is not known if the resorption of β -TCP and its conversion into bone occurs in a similar way as in animals, but based on the similarity in X-rays we hypothesize that in patients the same sequence of events takes place during the incorporation of the β -TCP scaffold as in animals. However, the only method to confirm this is histology.

This study concerns a radiological and histological follow-up examination of 16 patients with medial osteoarthritis of the knee treated with medial open wedge high tibial osteotomies (in one patient a bilateral procedure). The gap was filled with a wedge of β -TCP. At the time of the plate removal, a biopsy was taken through the implant site, which was processed for

histology. The process of incorporation is described and quantitative measurements of β -TCP resorption and new bone formation were carried out. The histology was correlated with the radiological classification of bone healing and related to the time frame between osteotomy and plate removal.

2. Materials and methods

In 16 patients (17 procedures; mean age at the time of taking the biopsy 43.6 ± 9.5 yrs), with a medial uni-compartmental knee osteoarthritis, the open wedge osteotomy was filled with a wedge of porous β -TCP ($\text{Ca}_3(\text{PO}_4)_2$ with 70% interconnected macropores with a size of 100–500 μm (Fig. 1A) and micropores of 1–10 μm (ChronOSTM, Synthes Biomaterials, Switzerland). One patient was operated on bilaterally. Preoperatively the degree of correction was determined on a standing long-leg film. The mean knee varus angle was 4.9 ($\text{SD} \pm 2.37$) with a range between 2° and 11° . The aim was a correction to 3° valgus creating medially based bone gaps between 5 and 14 mm. To preserve the correction, the osteotomy was fixed with a rigid angle stable plate (TomoFix, Synthes). Postoperatively the patients were allowed partial weight bearing of 10–15 kg for 6 weeks. Conventional AP and lateral radiographs were made immediately after the operation, at 6 weeks, 3 months, 6 months, and at 1 year postoperatively. The last X-ray before the removal of the fixation material was used for the radiological classification of bone healing.

A radiological classification system to monitor bone healing in open wedge HTO with resorbable substitutes was used [11]. In Phase 0 of this classification (Table 1), no remodelling takes place. In phase 1 there is a slight porosis of the bone. In phase 2 the β -TCP is denser and the interface between β -TCP and bone is blurred. In phase 3 the osteotomy is healed. In phase 4 the β -TCP is not longer recognizable as distinct entity, and in phase 5 the bone is normal again (complete remodelling).

Bone healing was studied histologically from bone biopsies taken during plate and screws removal between 6 and 25 months postoperatively (mean 14.7 ± 5.82 months). At that time, a core biopsy was taken from the medial $\frac{1}{4}$ part of the bone region where the β -TCP was implanted (Fig. 1B). The biopsy was long enough to contain both sides of the host bone. Serial non-decalcified sections along the long axis of all biopsies were made, which were stained with HE and Goldner. A TRAP-staining was applied for the visualization of

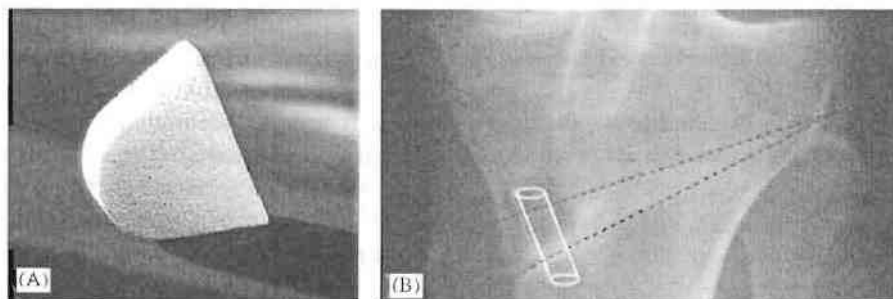


Fig. 1. (A). β -TCP wedge and (B) location of biopsy.

osteoclasts. Two biopsies could not be analyzed with TRAP-staining since they were broken after taking the biopsy or not processed according to the standard protocol. In all remaining biopsies the surface area of fibrous tissue, active incorporation (fibrous invasion of the β -TCP with osteoclast and osteoblast activity) and that of newly formed trabecular bone that still contained some β -TCP remnants at the location of the original β -TCP material, were estimated by two observers (HGT, PB). In areas with new bone formation the surface area of the remaining β -TCP and of bone was quantified with an automated image analysis system (AnalySIS) and expressed as percentage of the total region of interest.

3. Results

There were no complications during surgery or directly postoperative. Also, the clinical healing process was without complications in all cases (i.e. normal time until full weightbearing, no infection or non-union). Radiological follow-up showed complete consolidation at 12 months in all cases (Fig. 2). According to the radiological classification system all unions were classified as phase 4 or 5.

In 13 of 17 biopsy specimens, the original β -TCP was still visible in the biopsies (Fig. 3B and C). It had a

granular appearance and in HE sections pink-stained material inside the β -TCP indicated that material (fibrin or proteins) had penetrated into the micropores of the ceramic (Fig. 3B and C). Also small remnant areas of totally in bone-incorporated β -TCP were present. In six of these 13 biopsies also relatively larger area of the original β -TCP material was still present in the sections. In these biopsies active incorporation of the β -TCP was present. In four biopsies only totally remodelled bone was found without any remnants of β -TCP.

Particularly in the six biopsies with larger β -TCP areas, different stages of incorporation could be found. In the middle of the wedge, occasionally non-incorporated β -TCP with vascular fibrous tissue was found. More towards the osteotomy planes, the first bone formation was found as lamellar bone apposition onto the surface of macropores in the β -TCP blocks (Fig. 3C). At the same locations, small mononuclear cells were found in the pores between the β -TCP that did not stain for TRAP (Fig. 3B). These cells were always associated with red or dark green material in-between the β -TCP granules in the goldner sections. Apparently these mononuclear cells were osteoblasts that start to produce osteoid that subsequently mineralizes the micropores in the β -TCP material. Occasionally, in the

Table 1

Radiographic classification: phases in bone remodelling after open wedge high tibial osteotomy [11]

	Phase	Explanation
0	Direct postoperative	Haematoma
1	Vascular phase	Osteopenic bone, rounded osteotomy sites, clear distinction between TCP and bone
2	Calcification phase	Whitening of sites and blurred distinction between TCP and bone
3	Osteoblastic phase	Distinction between TCP and bone slightly visible, though healed osteotomy
4	Consolidation phase	Complete reformation, though osteotomy recognizable, no TCP
5	Complete remodelling	No sign of osteotomy

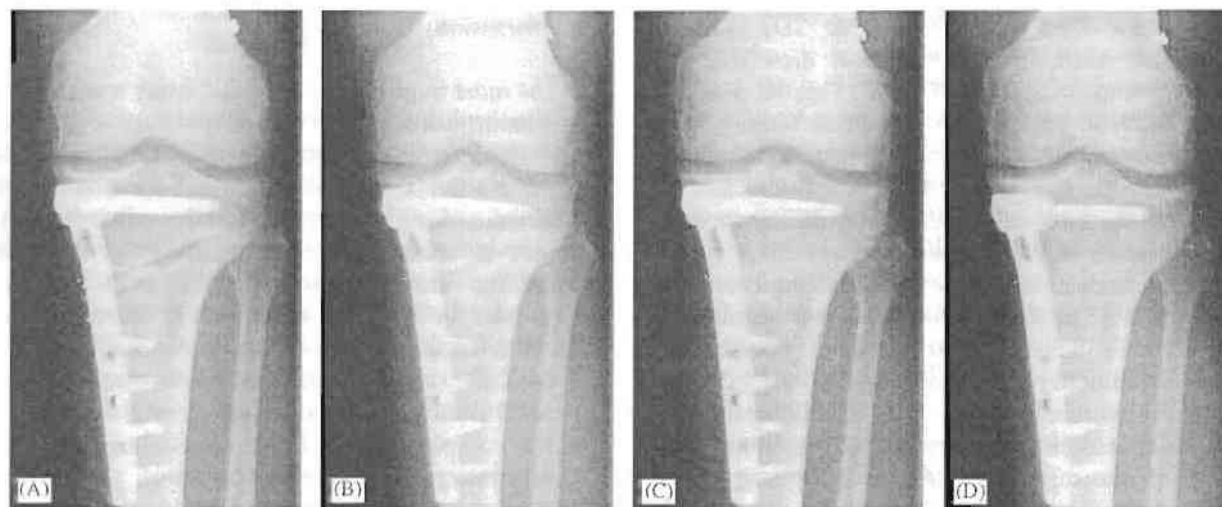


Fig. 2. Bone remodelling at different follow-up times after open wedge osteotomy filled with TCP. (A) at 6 weeks, (B) at 3 months, (C) at 6 months, (D) at 12 months. (Adapted from van Hemert et al. [11], with permission).

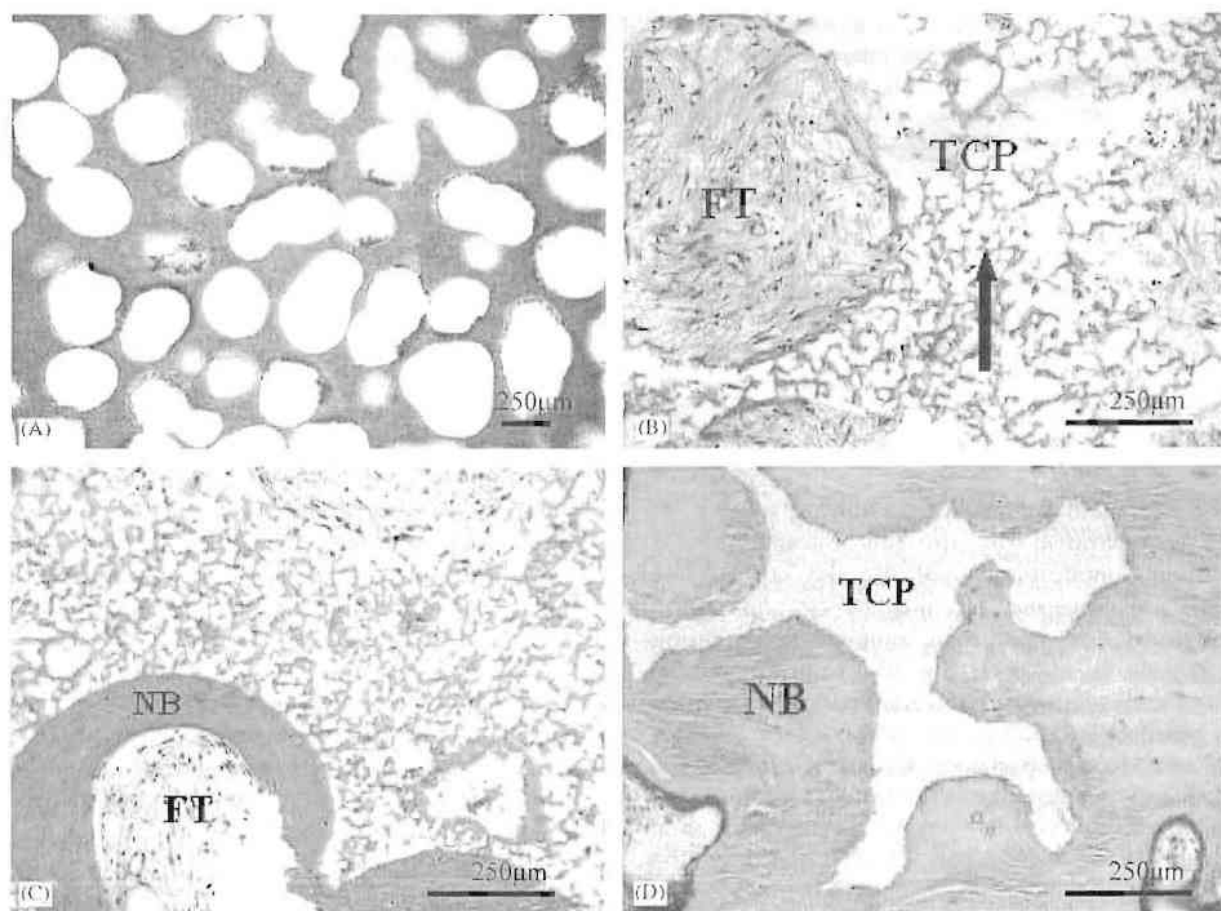


Fig. 3. (A) Thin section of β -CP material before implantation. (B) HE section of first stage of incorporation. Fibrous tissue (FT) has invaded the macro-pores in the TCP blocks and mononuclear cells have penetrated into the micropores of the TCP (arrows). (C) New lamellar bone formation in macro-pores of TCP. (D) Pores of TCP completely filled with new bone (NB). Scale bar: 250 μ m.

same locations, multinuclear TRAP positive osteoclasts penetrated into the micropores around the β -TCP granules and start to resorb the β -TCP (Fig. 4A and B). More close to the osteotomy side more bone apposition in the pores is found. Occasionally the pores are completely filled with bone (Fig. 3D). Locally abundant osteoclast activity is present dissolving the β -TCP remnants by surface erosion (Fig. 4C and D). The end result of this process is normal looking bone with only sparse remnants of β -TCP that are completely incorporated into new vital bone (Fig. 4E and F). No macrophages or giant cells were found during or after the incorporation of the β -TCP.

The mean surface area of active incorporation was 8.53% ($\pm 12.0\%$) in all specimens. The surface area of normal looking bone, which contained occasionally only sparse remnants of β -TCP was 77.1% ($\pm 21.1\%$) of the total area of the biopsy. The remaining tissue is fibrous tissue (13.8% ± 24.1). This means that more than 80% of the surface area of all biopsies consisted of bone or active incorporating bone. The surface area of remaining β -TCP in the regions with incorporated bone was 1.20% (SD 1.98) of the total measured

area. The surface area of bone in the bone region was 22.2% ± 11.6 , which is similar to normal human bone.

4. Discussion

The most important aim of this study was to compare the incorporation process in patients with that in animals. Particularly the assessment of bone substitute incorporation is very difficult on radiographs alone since the distinction between new bone and biomaterial cannot be made. Therefore histology is the ultimate proof for incorporation of bone graft replacement materials. As far as we know this is the first report on the detailed description of the incorporation of β -TCP in patients. Optimally, evaluation of biopsy specimens on several time points is necessary to give more insight in the completeness of bone remodelling, the type of incorporation process and the speed of biomaterial resorption and incorporation. In the patient population of the present study biopsies could only be taken after healing of the osteotomy.

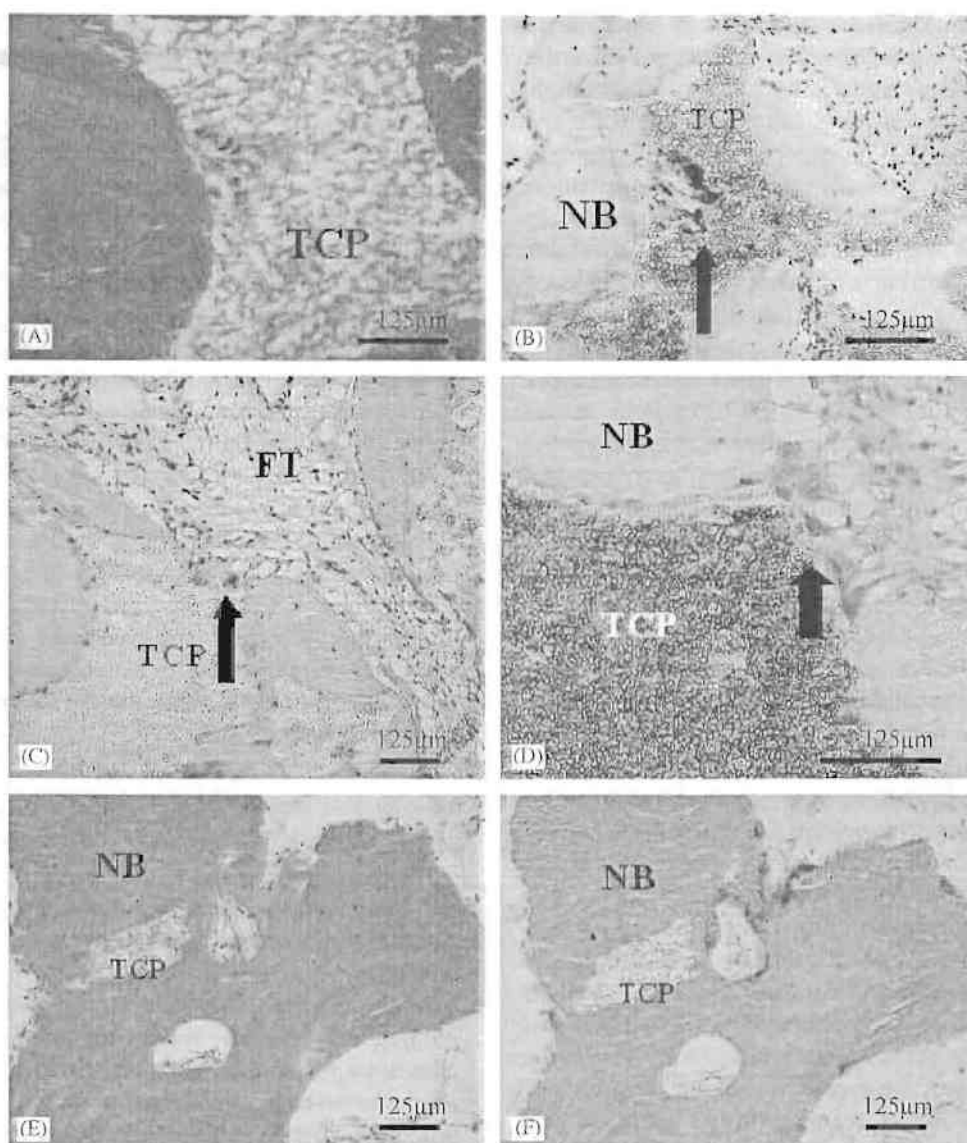


Fig. 4. (A) and (B). HE (A) and adjacent TRAP section of multinuclear osteoclasts penetrating into the TCP. (C) and (D) HE (C) and TRAP (D) section of surface erosion of TCP by osteoclasts. (E) and (F) HE and Goldner stained section of remnants of TCP incorporated into new bone. Scale bar: 125 μ m.

In various animal studies in which the incorporation of β -TCP was studied, it was described that the resorption of β -TCP and conversion into bone took place in a number of distinct phases [16,20]. Initially there is some resorption of host bone, probably due to the surgical trauma. This phase is followed by a phase in which apposition of bone occurs and the total density of bone plus β -TCP on X-rays is high. After about 6 months, resorption and remodelling of the β -TCP and new bone takes place, which decreases the total density again.

Although the biopsies were taken at considerable follow-up periods, different aspects of the incorporation process were found, although not in every specimen. Apparently the incorporation of the inserted wedge

takes a variable and in some cases considerable time. The variation may be related to patient related variables (e.g. age, smoker or not smoker), the thickness of the biopsy and differences in local environment (vascularity, loading). As the first biopsies in this study have only been obtained 6 months after the osteotomy, the local variation in incorporation enabled us to study also the first phases of incorporation.

Firstly there is invasion of fibro-vascular tissue in the large pores of the β -TCP. Probably simultaneous to this, there is preferential resorption of the necks linking the β -TCP microparticles. This might be caused by a process of chemical, cell mediated, dissolution as suggested by Zerbo et al. [29]. Next, bone apposition occurs on the inner surface of the large pores in the β -TCP.

Simultaneously mononuclear osteoblast like cells start to mineralize the microporous structure between the small β -TCP agglomerates. This phase is in animals associated with a sclerosing X-ray appearance. On the X-rays of human β -TCP incorporation this phenomenon is found during phase 2 of the radiological evaluation system [11]. Next multinucleated osteoclasts-like cells penetrate the β -TCP and start resorbing it by a cellular mediated process visible as decreasing β -TCP wedge density in phase 3 of the radiological classification. During the whole resorption process no macrophages or multinucleated giant cells are present confirming the good biocompatibility of the β -TCP. In later stages active resorption and bone apposition is responsible for the conversion of the β -TCP to healthy normal bone. This phase was not abundant in our biopsy material because of timing of removal of plate and screws. This is in general not performed before a phase 4 or 5 on the X-rays. In stage 4 some of the β -TCP may be present but in stage 5 the bone looks normal and indeed in the histology the β -TCP is then almost totally resorbed. What remains are small spots of β -TCP incorporated in bone at locations where no active remodelling takes place any more.

Egglı et al. described an average incorporation of 45% in the vertebral bodies of apes at 6 months and Buser et al. measured almost 70% bone matrix at six months in the mandibles of piglets [16,23]. Stoll et al. [17] studied bone incorporation after 12 weeks into a β -TCP implant in a cylindrical defects of 8.5 mm in the sheep tibia metaphysis and found 10% bone ingrowth when the implant was impregnated with fresh blood and 18% when the implant was impregnated with fresh bone marrow. In all cases, the expectation was that a further resorption of β -TCP and incorporation of bone would take place. Our study suggests that in human bone this expectation would have been met. Although the results show a high variation, in general a good resorption of β -TCP is seen and after 2 years even 95% complete incorporation and remodelling of bone.

Concerning the quantification of bone healing used in this study and the variability of results a few remarks can be made. First, the precise biopsy site is of interest. Because of the wedge-shaped β -TCP-filled defect, the site where the biopsy has been removed determines the quantity of β -TCP and bone seen in the biopsy. Although certain criteria are involved as to how and where the biopsy has to be taken (Fig. 1), it is not possible to obtain a biopsy from the exact same location in each patient. Also, the difference in the amount of correction between patients may have influenced the results. A higher number of degrees of correction means a larger defect that has to be filled and with that a larger surface area of β -TCP. Furthermore, the quality of the radiographs (overexposure versus underexposure) may have influenced its classification. Finally, smoking may

have played a role in bone healing. From several studies it has been found that smokers have significantly poorer (or worse) bone healing than non-smokers [30–35]. In this study, out of 16 patients, there were seven smokers. No difference between smokers and non-smokers could be demonstrated. Due to the fact that this involves a small study group, no conclusions can be drawn from this, however.

To obtain, as in animal studies, a complete and thorough overview of the process of resorption of β -TCP and conversion into bone, biopsies would have to be taken at earlier points in time (6 weeks, 3 months). In patients, however, the fixation material can only be removed, without loss of correction, after sufficient bone consolidation. In this study this was after at least 6 months and in phase 4 or 5 of the radiological classification. Only through biopsies taken at specific intervals after the osteotomy, a complete overview can be obtained. This would mean that the patient has to undergo a separate intervention with the fixation material still in situ, which poses medical-ethical problems prohibiting a complete overview in the current study structure.

5. Conclusions

In conclusion, although in only a few biopsies the first stages of incorporation were present, the overall conclusion is that the incorporation process of β -TCP in patients occurs similarly as in animals. The radiological classification system that was developed correlates with the histological findings. Most of the β -TCP is resorbed and completely incorporated and remodelled into new bone. Further research will be necessary to obtain more detailed information on the various phases incorporation of β -TCP in human bone though this will be difficult concerning the medical and ethical limitations.

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