The Knee 24 (2017) 1138–1145

Contents lists available at ScienceDirect

The Knee

ß-TCP bone substitutes in tibial plateau depression fractures

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A R T I C L E I N F O

Article history:
Received 16 December 2016
Received in revised form 20 March 2017
Accepted 27 June 2017

Keywords:
Bone graft
Calciumphosphate
ß-TCP
Tibial plateau fracture
Osseointegration

A B S T R A C T

Background: The use of beta-tricalciumphospate (ß-TCP, Cerasorb®) ceramics as an alternative for autologous bone-grafting has been outlined previously, however with no study focusing on both clinical and histological outcomes of ß-TCP application in patients with multi-fragment tibial plateau fractures. The aim of this study was to analyze the long-term results of ß-TCP in patients with tibial plateau fractures.
Methods: 52 patients were included in this study. All patients underwent open surgery with ß-TCP block or granulate application. After a mean follow-up of 36 months (14–64 months), the patients were reviewed. Radiography and computed-tomography were performed, while the Rasmussen score was obtained for clinical outcome. Furthermore, seven patients underwent biopsy during hardware removal, which was subsequently analyzed by histology and backscattered electron microscopy (BSEM).
Results: An excellent reduction with two millimeters or less of residual incongruity was achieved in 83% of the patients. At follow-up, no further changes occurred and no nonunions were observed. Functional outcome was good to excellent in 82%. Four patients underwent revision surgery due to reasons unrelated to the bone substitute material. Histologic analyses indicated that new bone was built around the ß-TCP-grafts, however a complete resorption of ß-TCP was not observed.
Discussion: ß-TCP combined with internal fixation represents an effective and safe treatment of tibial plateau depression fractures with good functional recovery. While its osteoconductivity seems to be successful, the biological degradation and replacement of ß-TCP is less pronounced in humans than previous animal studies have indicated.
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1. Introduction

Tibial plateau fractures represent a common fracture site and account for 1–2% of all fractures [1]. Usually they occur due to excessive axial loading combined with valgus/varus forces leading to a possible depression of the articular surface. To avoid secondary osteoarthritis, anatomic reduction of depressed joint fragments is the main goal in fracture treatment [2,3]. Thereby, the metaphyseal void beneath the articular surface following fracture reduction compromises the stability.

Filling options include autologous (autogenous), allogeneic bone grafting, or synthetic bone materials. Autologous bone transplantation (from the same individual) is considered to be the gold standard, however it has a limited supply, and rather high

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http://dx.doi.org/10.1016/j.knee.2017.06.010
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donor site morbidity [4]. Allografting relies on a sophisticated bone banking system and may elicit antigenic responses that delay osseointegration and carry the risk of infection transfer [5]. Furthermore, it was reported to have low initial mechanical stability in metaphyseal defects, and an inadequate long-term incorporation to the host bone [6].

To meet the requirements of biocompatibility, availability and biomechanical stability, bone graft substitutes have been developed with a trend towards osteoconductive materials, particularly calcium phosphates (CP) [7,8]. CP grafts have been in focus of several animal studies, where its complete resorption and remodeling was observed within 12–26 weeks [9].

There are only few studies that have analyzed the incorporation of CP bone grafts in human. In different bony defects, a partial replacement of CP but visible residues were seen after six months [10]. In open wedge high tibial osteotomy for medial knee osteoarthritis, ß-TCP incorporation was successful, however with visible remnants in most cases [11]. In a different study, histological assessment showed CP cement residues and signs of bone formation around the CP surfaces [12]. Therefore, the main problem seems to be a non-predictability of this resorption process and associated problems in biomechanical stability and bony surrounding.

Due to inconsistent findings between animal and patient use and the limited knowledge on the osseointegration of ß-TCP especially in patients with tibial plateau depression fractures, we analyzed the long-term results of ß-TCP bone replacement material in 52 patients with tibial plateau fractures. In particular, we used the Rasmussen score for clinical outcome, as well as radiography and computed-tomography (CT). A number of biopsies was taken during the hardware removal and subsequently analyzed by undecalcified histology as well as backscattered electron microcopy.

2. Methods

2.1. Patients

Within four years 184 patients with tibial plateau fracture were treated operatively at the Department of Trauma Surgery, University Medical Center Hamburg-Eppendorf, Germany. 52 patients with complex tibia plateau depression fractures (31 females and 21 males; mean age: 57 ± 17 years) were included in this study. All of them underwent open surgery combined with ß-TCP application. Patients with extra-articular fractures, open fractures, known cruciate ligament injuries or primary knee joint diseases were excluded from this study. The tibial plateau fractures were diagnosed by plain radiography (anteroposterior and lateral views) as well as computed-tomography (CT). All cases exhibited a depression of the tibial plateau of >5 mm requiring surgery. According to the AO/OTA (Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association) classification, 40 type-B (77%) and 12 type-C fractures (23%) were diagnosed (Table 1). Informed consent was obtained from all patients and presented data in line with the rules of the local ethics committee of the University Medical Center Hamburg-Eppendorf, Germany.

2.2. Beta-Tricalciumphosphate

The beta-tricalciumphosphate bone substitution material (ß-TCP, Cerasorb®, Curasan inc, Kleinostheim, Germany) used in this study was of synthetic origin and pure phase. The bone defect was completely filled with ß-TCP granules and/or block forms. ß-TCP granules (Cerasorb® M Granulate) were compacted without destruction of the granule structure. These granules of 1–2 mm diameter feature an interconnecting, open multi-porosity with micro-, meso- and macropores (5–500 μm) and a total porosity of approximately 65%. They are polygonal (i.e., irregularly shaped) and facilitate canting and interlocking in the defect cavity. ß-TCP block forms have regularly aligned, parallel macropores with diameters of 1000 to 1400 μm.

2.3. Surgical procedure and perioperative management

The AO/ASIF techniques for fracture reduction and fixation were used in all cases. Buttress plate osteosynthesis using Locking Compression Plates (LCP) with locking and conventional (non-locking) screws was performed in 43 cases (83%) and internal fixation with AO cancellous screws in nine cases (17%) (Table 2). Major metaphyseal voids were filled with ß-TCP blocks (n = 11) or a combination of blocks and granules (n = 18), minor defects were charged with granules exclusively (n = 23) (Table 2). Prior to their application, the scaffolds were mixed with the patient's blood (Figure 1 A, B). Following surgery, the leg was placed in a foam splint, and no active or passive motion was allowed for the first 24 h. No weight-bearing was allowed until six weeks after the surgery. The patients were allowed to walk with two crutches without weight-bearing of the affected leg and they were encouraged to gradually increase the range of motion using active exercises and a continuous passive motion device with the aim of

Table 1
Number of patients and respective fracture types according to AO classification (total 52 patients).

AO classification	1	2	3
A - extra articular	0	0	0
B - partial articular	1	18	21
C - complete articular	2	4	6

Table 2
Bone substitutes and surgical procedure type.

	Patients (n)
Bone substitutes	
ß-TCP granules	23
ß-TCP block	11
ß-TCP granules + block	18
Surgery	
Buttress plating	43
Screw osteosynthesis	9
Hardware removal	13
Biopsies	7

regaining complete extension and 90 degrees of flexion. Thromboprophylaxis with subcutaneous heparin was given in all patients during their stay in hospital and up to full weight-bearing.

2.4. Follow-up

Radiography and/or computed tomography (CT) were used at initial presentations as well as at follow-up in order to assess the degree of maximal joint depression and to check for possible delayed union or nonunion (Figure 1 C). Functional recovery was assessed using the Rasmussen score which includes pain levels, walking capacity, range of motion and stability (Score 0–30) [13]. Patients were furthermore asked to grade the overall treatment outcome in the affected knee (one very good and six insufficient) at time of follow-up. All complications were noted. From the 52 patients included in the study, 44 completed the follow-up examination.

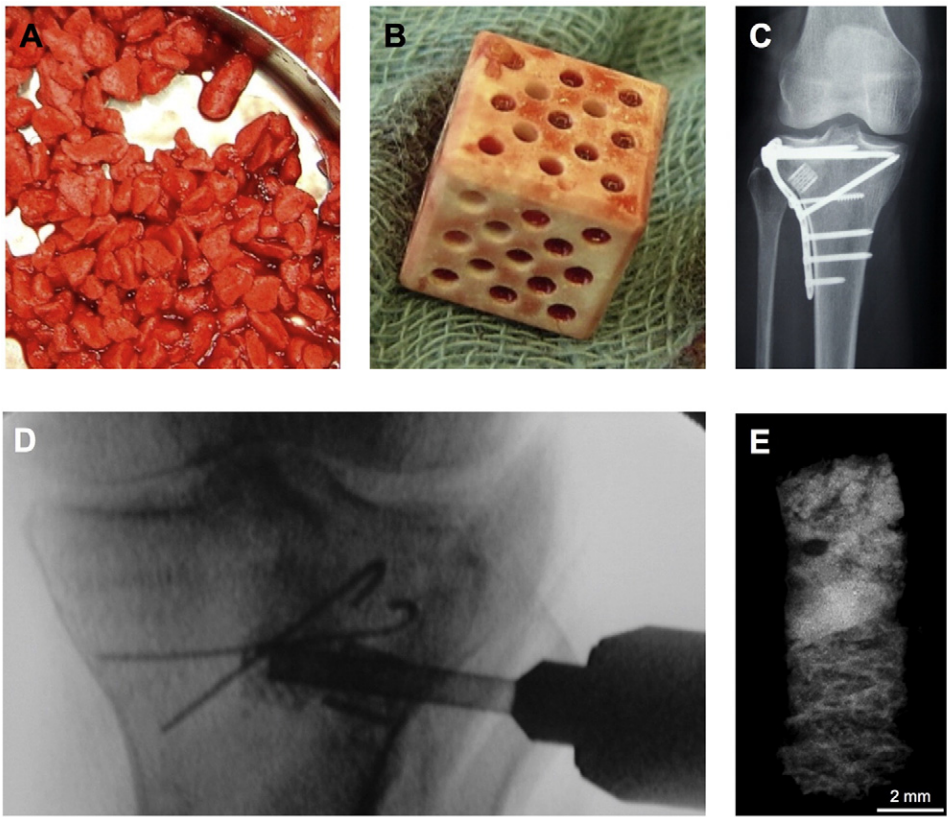

Figure 1. Beta-Tricalciumphosphate (ß-TCP) bone substitute material. (A) ß-TCP-granules. (B) ß-TCP-cube. (C) Antero-posterior radiography after application of a ß-TCP-cube in a AO B2 fracture. (D) Biopsy extraction with a liquid-cooled hollow drill (DBCS Diamond Bone Cutting System). (E) Contact radiography of a biopsy specimen.

2.5. Histology and backscattered electron microscopy

Routine-fashioned cylindrical biopsies were taken with a liquid-cooled hollow drill (DBCS-Diamond Bone Cutting System, Biomet Merck, Darmstadt, Germany) during hardware removal in seven patients (Figure 1 D). Earliest time of hardware removal was performed 12 months postoperatively. The extraction point was positioned under fluoroscopic guidance towards the center of the ß-TCP implantation site. The biopsies were approximately 10 mm in length and five millimeters in diameter (Figure 1 E). For undecalcified preparation specimens were fixed in 3.7% formaldehyde for three days, dehydrated, embedded in methyl methacrylate, and cut on a Microtec rotation microtome (CVT 4060E, Micro Tec, Walldorf, Germany). Subsequently, the 5-μm sections were stained by toluidine blue [14]. Morphological characteristics of the biopsies were analyzed with emphasis on the outcome of tissue-biomaterial interaction using a Zeiss microscope (Carl Zeiss Vision GmbH, Jena, Germany) as well as backscattered electron microscopy (LEO 435 VP; LEO Electron Microscopy Ltd., Cambridge, England) as described before by our group [15].

3. Results

Primary trauma mechanisms were falls (34%), car collision (20%), followed by bicycle accident (14%), motorbike accident (11%) and knee distortion 9%. With respect to local swelling, surgery was performed 8 ± 3 days after trauma. Mean duration of stay at the hospital was 18 ± 7 days.

3.1. Complications

There were no delayed or nonunions and no implant breakages. Early complications were seen in seven patients (15.9%). Among these seven cases, superficial wound infections were treated with antibiotics in three cases. Four patients underwent revision surgery, one patient with hematoma, one with intra-articular ß-TCP penetration, one with secondary loss of reduction, and one with loosening of the osteosynthesis (Table 3).

3.2. Clinical outcome

Eight patients had to be excluded at follow-up since they didn't show up to the arranged appointment. The remaining 44 patients were clinically and radiologically examined after a mean follow-up time of 36 months (14 to 64 months). The mean Rasmussen score was 25.6 ± 2.4 points and ranging from nine to 30 points indicating the good clinical outcome (Table 3). Patient's satisfaction score was not different with regard to age or fracture subtype (i.e., type B fracture 2.5; type C fracture 2.6) or age group. Four patients with a type B fracture presented with a lack of full extension up to five degrees and one patient up to 10°. Except one patient with a nearly stiff knee-joint, patients with type C fracture had no problems of knee extension at all. A knee-flexion of more than 90° was reached in 84% of the patients with a type B fracture (16% 61 to 90°; 37% 90 to 120°, 47% > 120°), and 79% of the patients with a type C fracture (11% 61°–90°; 22% 90 to 120°; 57% > 120°).

3.3. Radiographic analysis

The mean pre-operative joint depression of 10 ± 6 mm was significantly reduced to 0.9 ± 1.2 mm post-operatively. An excellent reduction with two millimeters or less of residual incongruity was achieved in 83% of the patients. Until follow-up, no further changes occurred. All fractures healed regularly. Complete resorption of ß-TCP did not occur in any case. Indeed, the boundary between the ß-TCP bone substitute and the adjacent bone appeared more blurred compared to previous images, but remnants of the ß-TCP could be identified in all patients (Figure 2 A–D).

Table 3
Clinical outcome and complications.

Outcome	
Rasmussen score	25.6 ± 2.4 (9–30)
Satisfaction score	2.3 ± 1.2
Complications	**Patients (n)**
Delayed union	0
Nonunion	0
Secondary loss of reduction	1
Implant failure	1
Superficial wound infection	3
Deep wound infection	0
Hematoma	1
Intra-articular ß-TCP penetration	1
Revision surgery	4

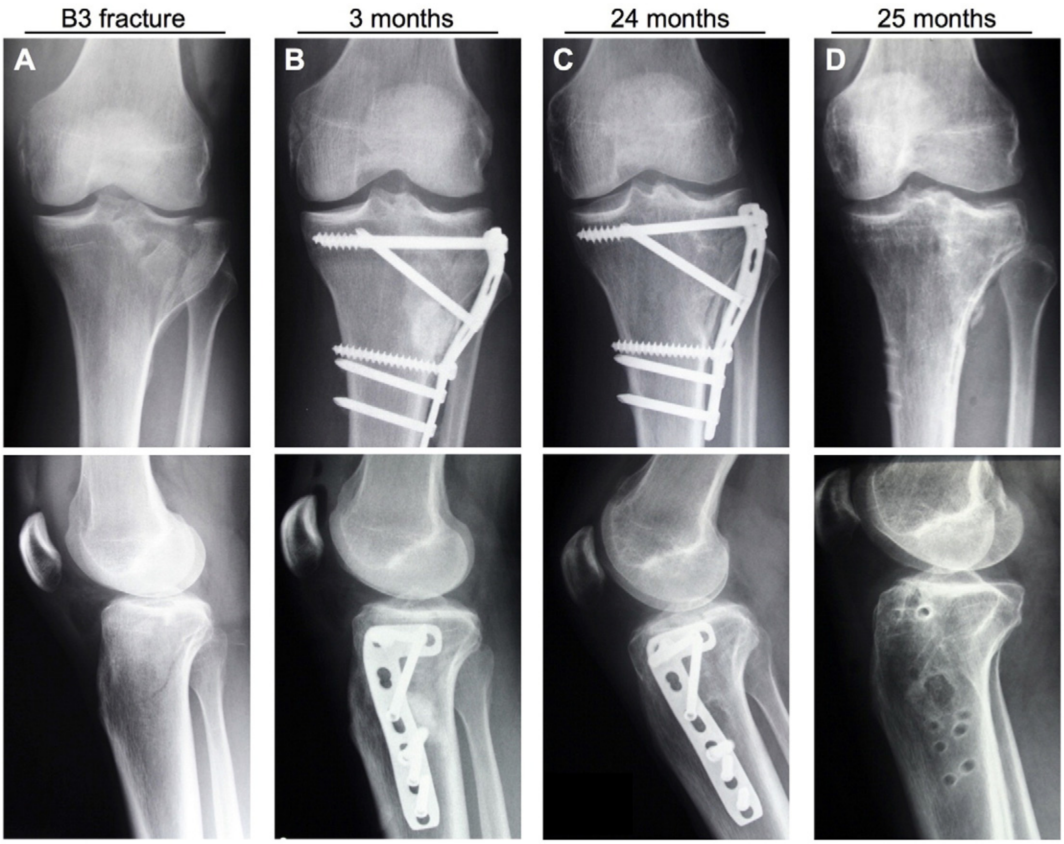

Figure 2. 47-year old patient with a B3 fracture. (A) Plain radiography before surgery. (B) 3 months following open reduction, buttress plating and application of the ß-TCP granules, (C) 24 month follow-up and (D) after plate removal.

3.4. Biopsies

The hardware was removed in 13 patients, while seven patients agreed to an additional biopsy (Table 2). No complications after hardware removal were observed. All specimens contained macroscopically and microscopically visible residues of the substitute material (Figure 3 A). The newly formed bone tissue in part surrounded the polygonal ß-TCP-grafts, indicating their osteoconductive function. There was no fibrous tissue layer between the ß-TCP-parts and the adjacent bone tissue and viable osteocytes were detected (Figure 3 B, C). Backscattered electron imaging (BSEM) revealed newly formed bone on the edges of the remaining ß-TCP material (Figure 4 A, B). Interestingly, only fragments smaller than 100 µm were completely incorporated into the bone matrix (Figure 4 C).

4. Discussion

Bony defects of the tibial plateau result from the trauma impaction, necessitating an open reduction combined with filling of these defects in most cases to restore the tibial plateau integrity. Ideally the bone graft ensures sufficient biocompatibility, osseointegration, biomechanical stability and degradation without any inflammatory response [8]. By complete remodeling of the bone substitute material, the development of an individual cancellous architecture, adapted to the local biomechanical forces, would thereby be desirable [16].

Bone substitute materials vary widely, while all of them have individual advantages and disadvantages. They range from hydroxyapatite [17], glass ceramics [18], synthetic biodegradable materials, polylactic acid and polyglycolic acid additions, demineralized bone [19], to calcium phosphates. Porous ß-TCP, as used in this study, is a ceramic bone substitute with material properties much like the inorganic phase of bone, which constitutes 60–70% of human bone [20].

Several animal experiments with respect to biodegradation, incorporation and remodeling of ß-TCP have been performed, but the results vary widely with respect to the experimental set-up, examined animals and mechanical loading pattern of the implanted ß-TCP. In fact, a good osteoconduction of ß-TCP in a sheep model for anterior spinal fusion and a full osseointegration and gradual resorption of ß-TCP plugs into vertebral bodies of baboons has been found [21]. In canine long bone defects, a

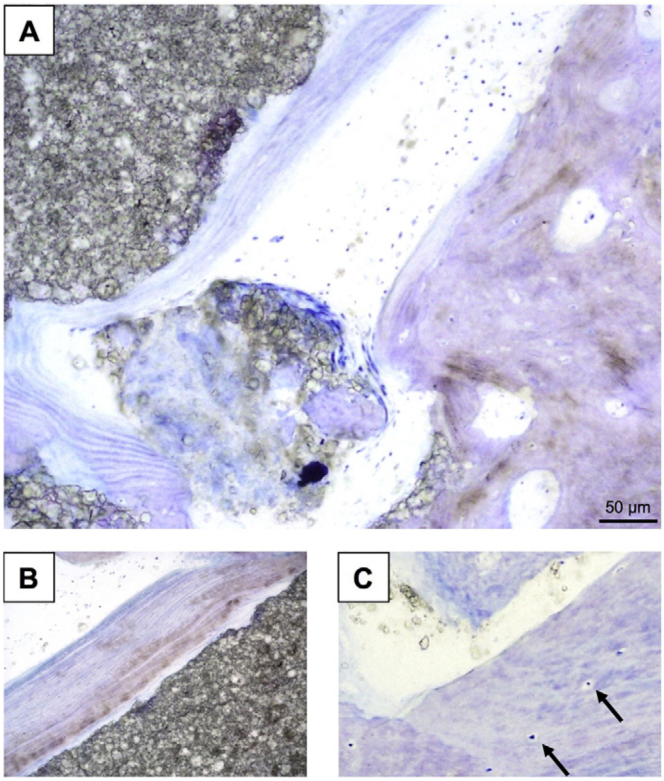

Figure 3. Histological findings, toluidine blue staining. (A) The residual ß-TCP was clearly visible and surrounded by bone. (B) The newly formed bone had a direct contract with the bone substitution material. (C) Viable osteocytes were found in the bone surrounding the ß-TCP remnants.

quick resorption of ß-TCP with only small amounts of residual graft material was observed after 24 weeks [22]. By contrast, in drill hole defects no resorption or remodeling was found in sheep and rabbits [23]. Furthermore, incomplete degradation of ß-TCP was observed in a weight-bearing sheep model [24].

In humans, van Hemert et al. used a radiographic rating system to monitor bone healing with ß-TCP granules or wedges in open wedge high tibial osteotomies. They observed a complete resorption of ß-TCP in 85% of the procedures at one-year post-operation, and they found no difference in bone healing and remodeling comparing the usage of granules or wedges [25]. For biphasic calcium phosphate (BCP) substitutes, a significant longer resorption-rate was reported [26]. This could be caused by a higher amount of hydroxyapatite in BCP (60%) as in ß-TCP (40%) [20]. However, other studies using ceramic biphasic bone substitutes in tibial plateau fractures outlined complete resorption after three to eight months in radiographic follow-up examinations [27].

In our patients, complete resorption of ß-TCP did not occur. Albeit very residual, we radiologically identified ß-TCP remnants in all cases. This was independent from whether block forms and/or granules were implanted. Our radiological findings were confirmed by the histological and BSEM-analyses, which indicated that complete resorption was not achieved, however the newly formed viable (osteocyte-containing) bone was in direct contact with the ß-TCP remnants; and particles under 100 μm in diameter were successfully incorporated.

In general, the functional outcome following tibial plateau fractures varies significantly. At follow-up, 82% of our patients had excellent or good results as assessed by the Rasmussen score. This is in line with previous findings, that have equally shown good functional results, i.e., twelve months after treatment of tibial plateau fractures with high strength injectable calcium phosphate [28] or ß-TCP, respectively [29]. Furthermore, the absence of a deep infection or osteomyelitis in the patients examined in this study is of main interest, since it was demonstrated in vitro before, that larger, non-phagocytosable ß-TCP particles might induce inflammatory and cytotoxic reactions [8]. In this study, residual ß-TCP was not accompanied by functional limitations or inflammatory responses until up to 74 months follow-up (mean 36 months). Long-term complications are not documented so far, but are highly unlikely given the positive results of our analysis.

There are several studies investigating the subsidence of the tibial plateau after CP-cement augmentation [30]. CP-cement repairs have been reported to have significantly higher fatigue strength and ultimate load than autogenous bone graft repairs [31]. Furthermore, augmentation with CP-cement prevented subsidence of the fracture fragment and maintained articular congruency [32]. In the present study, tibia plateau depression was successfully treated surgically by ß-TCP bone substitutes to a minimum residual of 0.9 ± 1.2 mm. Only in one case we observed a secondary loss of reduction with depression of the tibial plateau.

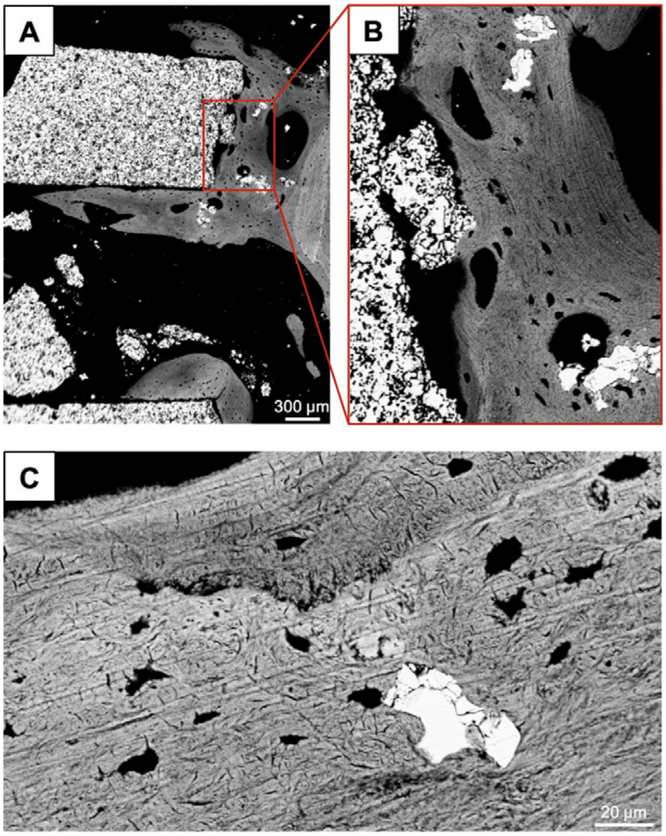

Figure 4. Backscattered electron microscopy (BSEM) analysis of the osseointegration of ß-TCP. (A) BSEM-view of a biopsy. (B) Interface between ß-TCP and bone. (C) Incorporated ß-TCP remnant.

However, this patient had loaded the operated leg with full weight accidentally. Overall, this means that our results are comparable to previous studies with CP-cement.

Future investigations including randomized controlled trials should be conducted to evaluate the functional and histological outcomes of ß-TCP in comparison to other bone substitute materials (i.e., Rasmussen score, level of bone substitute integration and resorption). However, depending on the examined endpoints and effect size, more than one hundred patients would have to be included in these studies in order to achieve enough statistical power.

Although a relatively small number of biopsies was obtained in this study, the number was sufficient to outline the excellent clinical outcome and biocompatibility of ß-TCP to bone as well as the absence of inflammatory reactions. As we found only incomplete remodeling of ß-TCP, it is important to illustrate the differences to results from animal experiments.

5. Conclusions

Taken together, this study illustrates that ß-TCP blocks and/or granules combined with internal fixation represent an effective and safe treatment of displaced tibial plateau fractures with good functional recovery. ß-TCP bone substitutes represent a promising alternative to autografts and allografts. The degradation and replacement of ß-TCP is less pronounced in patients than in previous animal studies. Further studies and quantitative histomorphometric analyses have to be carried out for further insights into the characteristics of the osseointegration and remodeling of ß-TCP under conditions of a human fracture site.

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