Histomorphological, histomorphometrical and biomechanical analysis of ceramic bone substitutes in a weight-bearing animal model

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It was the purpose of this investigation to prove the biomechanical properties, the osteoconductive capacity and the degradation rate of α tricalcium phosphate (α TCP), a neutralized glass ceramics (GB9N) and a composite material (GB9N + copolymers).

In a weight-bearing animal model six substitutes each were implanted in the medial tibial head of the right lower leg of adult Merino-sheep in a standardized surgical technique. After nine months the implants were harvested and prepared for histomorphological and histomorphometrical investigations (undecalcified Masson Goldner staining). For additional biomechanical testing of the specimens, non-operated bone blocks from the contralateral tibia as well as native implants served as controls.

No significant differences for the maximum fracture load as well as for the yield strength were detected between harvested specimens and bone blocks from the contralateral tibia. However there were marked differences to ceramics that were not implanted.

All substitutes showed osteoconduction, leading to a continuous ingrowth of new formed bone. However in the composite material soft tissue could be identified within the scaffold and there were signs of ongoing bone remodeling, nine months after implantation.

The bone per tissue volume of α-TCP in conjunction to new bone (= percentage of trabecular bone volume plus percentage of residual substitute) was higher than for GB9N and the composite material. Nine months after implantation the percentage of residual α-TCP was 48%, it was 32% for GB9N and 28% for the composite.

The intention of further studies should be to accelerate the degradation rates of substitutes and to improve biomechanical properties of implants by either modifying the chemical composition or combining materials with agents as, e.g. growth factors.

1. Introduction

The repair or replacement of bone defects in trauma or tumor surgery, implant loosening and spinal fusion is a common indication for orthopedic surgeons. According to several investigations bone transplantation is necessary in Germany in more than 100 000 surgical cases per year [1].

Autogenic transplantation is associated with certain risks for the patient and with limited availability. Allogenic transplants require screening and sterilization techniques as they bear the risk of transmission of infectious diseases [2, 3]. The morbidity associated with autogenic bone grafts and the concerns regarding the transmission of infections by the use of allografts have been the mainspring for the development of synthetic bone substitutes.

Several investigations have shown that bone defects can be treated sucessfully with a variety of synthetic materials like hydroxyapatite [4], calcium phosphate [5–9] and glass ceramics [10], synthetic biodegradable materials like polylactic acid [11], copolymers of polylactic acid and polyglycolic acid [12, 13] or demineralized bone [14–17].

All substitutes have to fulfill certain histological and biomechanical requirements. An almost complete bone remodeling should take place to allow the development of an individual cancellous architecture, which is adapted to the local biomechanical forces [18]. Hydroxyapatite ceramics show no appreciable resorption, as they are long-lasting implants. Tricalcium-phosphate (TCP) however is degraded continuously by phagocytosis and solubility [4, 19] but it is often too slow in comparison to the desired bone remodeling. Glass ceramics show a high solubility, but have a limited biocompatibility [10] due to their initial high basicity [20]. Neutralized glass ceramics with a modified surface structure show an

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improved biocompatibility *in vitro* [21] and *in vivo* [20]. Another possibility to minimize the initial basicity is the combination with resorbable organic polymers like polylactic acid or polygicolic acid in a composite [11, 13, 22]. These polymers show a degradation in an acid environment. However it is not clear so far, if the increased solubility of so called ''neutralized ceramics'' *in vitro* is going along with a decreased time of resorption *in vivo*.

It was the purpose of our investigation to test the osteointegrative capacity, the degradation rate and biomechanical properties of three different synthetic bone substitutes: pure α tricalcium phosphate (α-TCP), a neutralized glass ceramics (GB9N) and a composite material (GB9N + polymer) in a weight-bearing animal model.

2. Materials and methods

2.1. Material properties

Pure α tricalcium phosphate (α-TCP), a neutralized glass ceramics (GB9N) and a composite material including GB9N and copolymers were used within this investigation. Material properties including chemical composition are presented in Table I. Porosity of substitutes ranged between 50 and 60%. Macro and micro pore size were respectively 600 μm and 5 μm within an interconnective pore system. The *in vitro* solubility of GB9N was at least four-fold higher when compared to alpha-TCP. All substitutes were provided by BIOVISION, GmbH, llmenau, Germany and available as sintered triangular specimens.

2.2. Implantation technique

Eighteen healthy Merino sheep were used in this study. All animals were parasite-free and were skeletally mature. Within this weight-bearing animal model six substitutes each were implanted in the medial tibial head of the right leg in a standardized surgical technique. The sequence of substitutes used for implantation was determined by chance. The study protocol was approved by the German Regierungspräsidium (Tübingen, no. 612) and followed national regulations for the care and use of animals.

With the animal under general anesthesia, a defect was drilled 3 mm under the articular surface of the tibial plateau in the size of 6 × 12 × 24 mm by using a special guiding device. The triangular substitutes were positioned into the wedge-shaped defects by press-fit technique. The operative wound was then closed in layers and the animal was brought back to its box. All sheep were able to bear full weight within two days after operation. No significant complication like wound infection or fracture happened within the study period.

After nine months animals were killed and both tibias were excized. The former implant site and the corresponding area of the left tibia were identified by X-ray and macroscopically and divided into two parts for histomorphological respectively histomorphometrical as well as for biomechanical investigations.

2.3. Histomorphological and histomorphometrical analysis

After fixation in formaline, specimens were processed with the use of graded concentrations of alcohol and then embedded in methylmethacrylate. Undecalcified sections of 100 μm were prepared using an EXACT diamond saw (Grünewald GmbH, Laudenbach, Germany) and stained with trichrome Masson–Goldner to visualize new bone formation.

Within a semiautomatic analysis system (LEITZ ASM 68K), histomorphometrical data were gained. The percentage of new bone that had formed in the defect was measured as well as residual ceramics. The bone per tissue volume (%) was determined by adding both parameters and relating them to the visual field in the microscope.

2.4. Mechanical analysis

Cubes of 6 × 5 × 3 mm from each specimen were sawed and tested in a compression test (material testing machine, 1445 Zwick, Ulm, Germany). From the load displacement curve the maximum fracture load and yield strength were determined. The yield strength is that force, in which the increase of the line of force reaches zero for the first time.

Results of the specimen were compared to bone harvested from the same region of the contralateral tibia as well as to ceramics that were not implanted.

2.5. Analysis of data

Data were collected in a Microsoft Excel data base, Version 7.0 and results are presented as means and standard deviations. The significance of differences between samples was determined using the Student's *t*-test for paired samples.

3. Results

3.1. Histomorphological investigations

α-TCP and the glass ceramics showed osteoconduction with homogenous bone formation throughout the

TABLE I Material properties of α-TCP, GB9N and composite (GB9N/RG585) (provided by BIOVISION GmbH, Ilmenau Germany)

Substitute	α-TCP	GB9N	Composite
Porosity	58–63%	50%	50%
Pore size	200–1000 μ/5μ	200–600 μ/5μ	200–600 μ/5μ
In-vitro solubility	1	4	—
Chemical composition	$[Ca_3(PO_4)_2]$	43.2%P_2O_5; 35.9%CaO; 1.1%SiO_2; 1.1%MgO; 7.5%Na_2O; 10.7%K_2O	45% GB9N + 55% Poly (D,L-lactide-co-glycolide)

implants and osseous trabeculae bridging and connecting the defect side. The surface of the new formed bone was lined with osteoblasts. Osteoid formation resulting from actively synthesizing osteoblasts was seen only very rarely. Residual matrix of ceramics, degradated to small particles layed between the osseous trabeculae. Multinucleated foreign-body giant cells could not be identified within the 100 μm sections and there were no signs of infection (Figs 1, 2).

The composite was osteoconductive as well with trabeculae bridging and connecting the defect side. However apart from bone, islands of connective tissue were identified (Fig. 3) with collagen fibers visualized under polarized light. Nine months after implantation, there were still signs of high osteoblastic activity with active cells lined to the surface of the trabeculae and synthesizing osteoid. Residual ceramics degraded to small particles were seen between the trabeculae and in the cytoplasm of multinucleated foreign-body giant cells.

3.2. Histomorphometrical investigations

Nine months after implantation the bone per tissue volume of α-TCP and new formed bone was higher, compared to GB9N and bone respectively the composite in conjunction to new formed bone. Results were significantly higher ($p \leq 0.05$) when compared to cancellous bone of the controls (Fig. 4).

Degradation rates of substitutes were higher for the glass ceramics and for the composite than for α-TCP. Nine months after implantation the percentage of residual matrix was 32 and 28% for GB9N and the composite. For α-TCP it was somewhat higher, with 48%.

3.3. Mechanical analysis

The maximum fracture load and yield strength of GB9N and the composite showed no significant differences to controls (Table II). Biomechanical properties of α-TCP were different compared to the controls with a higher

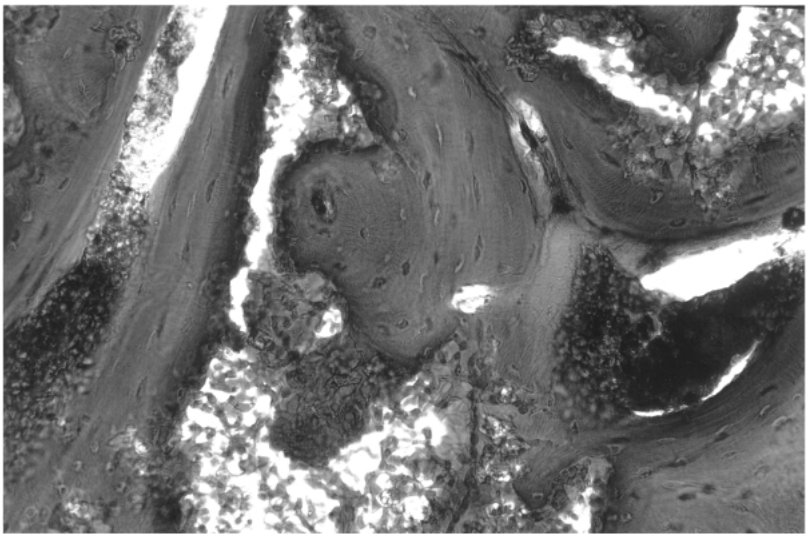

Figure 1 Histological section (40-fold magification) of α-TCP nine months after implantation shows newly formed trabelular bone bridging the defect as well as residual ceramics. No significant signs of ongoing bone remodeling were found.

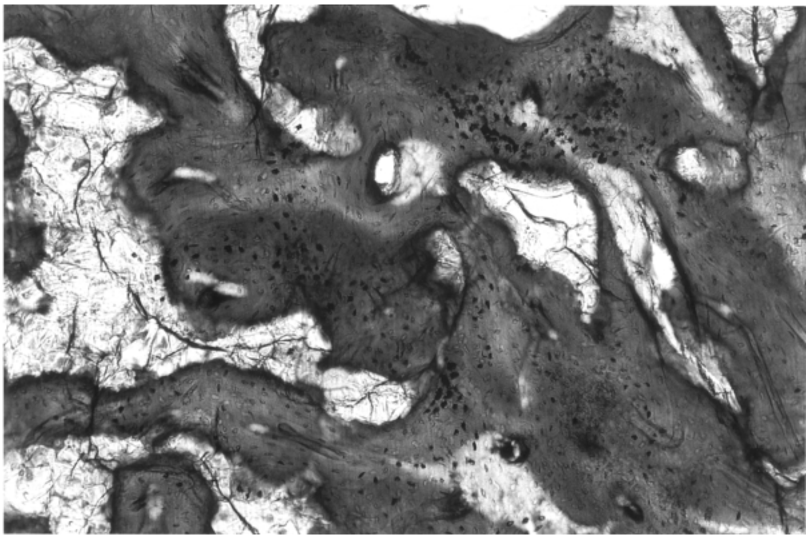

Figure 2 Histological section of GB9N (40-fold magification) nine months after implantation. There was newly formed trabelular bone bridging the defect as well as residual ceramics. No significant signs of ongoing bone remodeling were found as well.

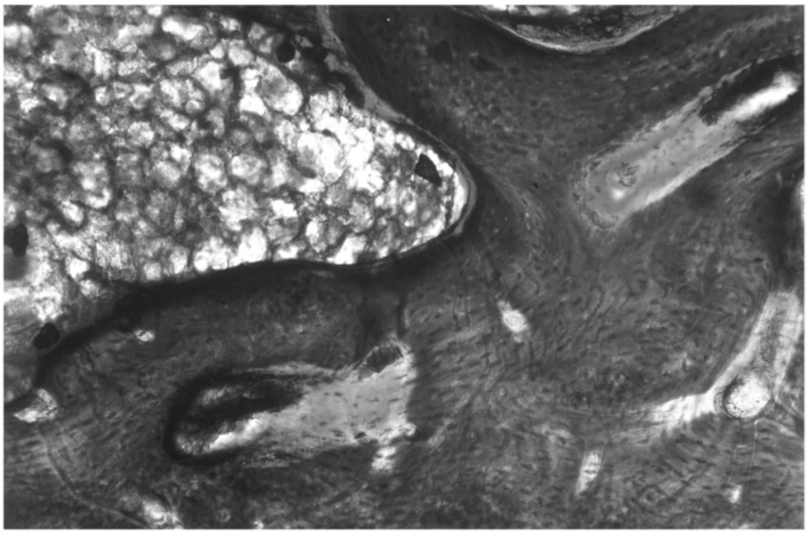

Figure 3 Histological section of the composit (GB9N + copolymers) nine months after implantation (40-fold magification). Apart from new bone formation, fibrous connective tissue is identified (yellow-orange areas). Broad areas of osteoid as signs of ongowing osteoblastic activity are seen.

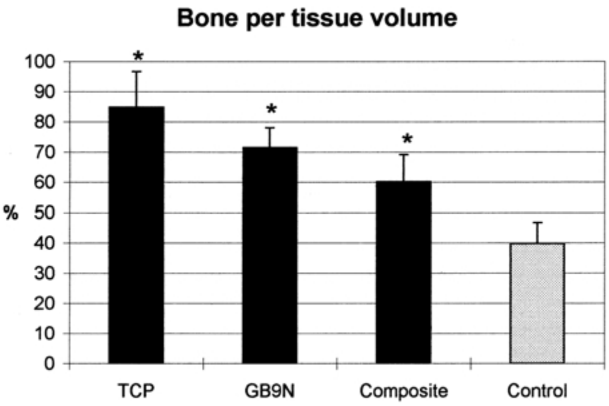

Figure 4 Bone per tissue volume (%) of new formed trabecular bone in conjunction to rests of implants for α-TCP; GB9N and composite. Values were significantly higher when compared to controls ($*p \leq 0.05$).

maximum fracture load and yield strength. Results for native ceramics were significantly lower (Table II).

4. Discussion

The need for synthetic bone substitutes as alternatives to autogenic or allogenic bone transplants bridging bone defects is recognized [1]. Within this *in vivo* study three different synthetic bone substitutes were tested on their histomorphological, histomorphometrical and biomechanical properties using a weight-bearing animal model.

Histomorphological investigations have demonstrated the osteoconductive capacity of all three synthetic substitutes with bone formation bridging the defect site. However, the trabecular geometry after partial implant degradation is different to the cancellous structure of the controls. That is expressed in the bone per tissue volume as well, with significantly higher values within the harvested implant in comparison to bone of the contralateral side. Apart from new formed osseous trabeculae remnants of partially degraded ceramics were found as well. Other investigators like Cameron *et al.* [23] noticed the ability for osteoconduction of ceramics in their experiments as well. They found direct apposition of bone on implant surfaces at 3–6 weeks after implantation. They concluded that at least TCP would already be suitable for clinical use.

Nine months after the implantation of α-TCP and GB9N no significant signs of ongoing bone formation were detected. Although the surfaces of the trabeculae were lined with osteoblasts, osteoid formation resulting from actively synthesizing cells was seen only very rarely. There were no signs of inflammation and only some multinucleated foreign-body giant cells could be detected within our undecalcified sections.

While the exact mechanism of biodegradation remains unclear, some researchers suggest that in an acid environment synthetic substitutes would dissolve *in situ* [24]. Eggli *et al.* [25] noted osteoclast like cells attached to TCP and suggested a cellular breakdown by macrophages. However some materials remain in the defect site for extended periods [26] and appear incorporated within the new bone structure [27]. Our analyzes demonstrated an inclomplete dissolution respectively degradation of substitutes as well.

Some histomorphologic differences were found for the composite material used within this investigation. There were still signs of a high osteoblastic activity with active osteoblasts lined to the surface of the trabeculae and synthesizing broad borders of osteoid. Furthermore, with the use of polarized light islands of fibrous connective tissue were identified apart from osseous trabeculae. We regard these regions as being the remnants of the copoylmers which are known to provide better biomechanical properties in the beginning but then being degraded fast by solution or phagocytosis and leaving defects which are filled by fast proliferating tissue, as fibrous connective tissue. We do not believe that the ceramic component of the composite material is responsible for these areas.

Other investigators had problems when using polyglycolids or polylactids as well. Silverberg *et al.* [28] described a foreign body response when using polyglycolic tubes, which appeared to inhibit initial bone formation until it was resorbed. Tencer *et al.* [29, 30]

TABLE II Results of biomechanical investigations as maximum fracture load (N) and yield strength (N/mm2) of harvested implants, controls and native ceramics prior to surgery. GB9N and the composite showed no significant differences to controls, whereas values for α-TCP were significantly higher when compared to the controls ($p \leq 0.05$)

	Maximum fracture load F_{max} (N)	Yield strength (N/mm^2)
1. TCP (harvested)	2047.1* (391.8)	80.5* (18.6)
2. Control to 1.	1119.4 (510.9)	45.4 (18.4)
3. GB9N (harvested)	1441.7 (699.7)	53.9 (23.3)
4. Control to 3.	1173.2 (700.7)	38.7 (18.4)
5. Composite (harvested)	1280.9 (599.3)	46.7 (22.8)
6. Control to 5.	1311.3 (313.8)	45.5 (9.9)
TCP (native)	24.7 (9.2)	0.9 (0.3)
GB9N (native)	46.6 (15.0)	1.7 (0.7)
Composite (native)	—	28.0 (6.3)

using polylactic acid in their composite material reported that polymers would obstruct pores and bone would have difficulties to penetrate the substitute. Once the polymer would be degraded, new bone would form in place.

The mechanical properties of our harvested implants were good. Maximum fracture load as well as yield strength were at least as high as those of the controls taken from the contralateral tibia. Ceramics prior to surgery were measured as well. The mechanical properties of these indicate stress tolerances much lower than for the harvested implants and do not allow immediate postoperative weight-bearing. The increased stress tolerances after nine months can be regarded as the result of the new formed trabecular bone bridging the defect side.

Further investigations on synthetic bone substitutes have to improve the balance between implant resorption and bone remodeling which is still desirable under biological and biomechanical aspects, e.g. by combining substitutes with certain agents like growth factors. That might allow an earlier weight bearing after implantation of bone substitutes. Apart from accelerating the degradative process, local osteoinduction should be inforced to cover the biomechanical defencies of substitutes in the beginning.

Acknowledgment
This study was funded by the German Ministry for Research and Technology (grant number JP05SB).

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Received on 22 June
and accepted on 24 December 2000