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***In Vivo* Evaluation of β -TCP Containing 3D Laser Sintered Poly(ether ether ketone) Composites in Pigs**

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ABSTRACT: The aim of this study was to produce an individually shaped medical implant from a 3D database and to evaluate the biological behavior of a laser sintered poly(ether ether ketone) (PEEK) implant with incorporated osteoconductive bone materials in porcine bone defects. Laser sintered PEEK samples containing β -tricalciumphosphate (β -TCP) were implanted into critical size defects in the frontal skull of ten pigs. Compression moulded pure PEEK was used as a reference material. The bone-implant interface was histomorphometrically analysed after 6, 12, and 24 weeks. Histomorphometrical evaluations after 24 weeks revealed that the superficially located β -TCP was in contact with the surrounding bone, whereas the other groups were fibrous encapsulated.

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Figures 1, 4 and 5 appear in color online: <http://jbc.sagepub.com>

Interfacial shear strength was significantly higher for the β -TCP containing group in comparison to the compression moulded PEEK group ($p = 0.004$) and the laser sintered PEEK group. The laser sintered PEEK implants seem to be attractive as bone substitutes for reconstructive surgery due to their individually constructed 3D shape and biocompatibility.

KEY WORDS: tissue engineering, poly(ether ether ketone) (PEEK), laser sintering, β -TCP, *in vivo*, bone substitution, sintered PEEK biocompatibility, 3D shaped implants, individually shaped implants, bone reconstruction.

INTRODUCTION

The reconstruction of large bone defects from tumor surgery, malformation and accidents is still a major problem. Autologous cancellous bone is regarded as the gold standard for bone grafting. The well-accepted advantages of autologous bone grafts, such as good and fast osseous integration, are opposed by disadvantages like donor site morbidity and limited availability. With advances in polymer technology, synthetic graft materials are possible alternatives as medical implant materials.

Poly(ether ether ketone) (PEEK) is a semi-crystalline thermoplastic, that has sufficient strength and stiffness with long-term biostability. Due to its mechanical properties, PEEK is used in a wide range of medical applications like spinal cages and fracture fixation plates [1]. These medical devices are produced with conventional manufacturing processes like injection moulding or machining. Considering the complex geometry of bone defects, a more amenable manufacturing technique is needed. Due to its easy fabrication into very complex 3D anatomical structures, laser sintering seems to be an attractive production technology. Based upon digital computer data (DICOM datasets), it is suitable for direct fabrication of single parts with an individual shape, such as implants for medical application.

A major difficulty encountered with polymer implants is to achieve a secure attachment between the implant and the surrounding bone [2]. A non-degradable polymer, such as PEEK, is generally encapsulation after implantation into a living body by fibrous tissue that isolates the implant from the surrounding bone [2]. In order to improve the performance of polymer implants it is necessary that the implant surface is capable of bonding to living bone without formation of fibrous tissue.

Calcium phosphate ceramics like tricalciumphosphate or hydroxyapatite have good bone binding properties due to their chemical similarity

to the bone mineral [3,4]. It offers the advantage of a more secure attachment upon implantation than conventionally manufactured implants. The use of high-performance polymers may reduce the risk of bone loss due to the mismatch in Young's modulus of elasticity and stiffness between implant and bone. Consequently, this study aims to determine the potential of the rapid-prototyping laser sintering technique to produce 3D, individually shaped, medical PEEK implants for use in reconstructive surgery. The tissue behavior of laser sintered 3D PEEK implants and the effect of incorporated β -TCP on bone formation were investigated in a critical size bone defect in pigs.

MATERIALS AND METHODS

Implant Material

PEEKTM 150 PF powder (Invibio Ltd., Thornton-Cleveleys, Lancashire, UK) was used. This material has the lowest melt viscosity of all the commercially available PEEK powders and best suited for the laser sintering process [5]. The PEEK powder exhibits irregular, edged particles and so does not flow smoothly. By adding 1 wt% carbon black (Degussa AG, Frankfurt, Germany), the powder flow was greatly improved and the application of the powder in the sintering machine was possible. To generate a thin, homogenous powder layer with a thickness between 100 and 150 μm , it was necessary to sieve the powder below a particle size of 63 μm (AS 200, Retsch GmbH, Haan, Germany, sieve shaker).

For one test series, in addition to carbon black, the PEEK powder was compounded with 10 wt% β -tricalciumphosphate (β -TCP, Merck, Darmstadt, Germany) a clinical appliance used as a bone substitution material [6]. The β -TCP powder used was classified as dry and purest, following European pharmaceutical guidelines (E141, Ph Eur, British Pharmacy).

Implant Design and Fabrication

Compression moulded pure PEEK specimens [PEEK-CM] were used as the control material. Cylindrically shaped implants 6.7 mm high and 10.0 mm in diameter were used. These implants were pressed at 375°C in a vacuum chamber and subsequently machined to the desired geometry.

A modified EOSINT P 380 sintering system (EOS, Krailing, Germany) was used to generate the laser sintered test specimens as described [7,8]. The layer thickness for the laser sintering of test specimens was 0.15 mm. The applied powder layers were preheated up to 334°C and then laser

treated with a laser power of 10 W, a scan velocity of 4.5 m/s and a hatch distance of 0.2 mm [8]. The mechanical properties of laser sintered PEEK have been described previously [9].

To manufacture an individually shaped medical implant polymer by laser sintering the following steps were carried out. The 3D data of the individual defect situation was recorded by computer tomography (Somatom Plus, Siemens, Erlangen, Germany). The recorded data consisted of a DICOM-dataset which is the typical format for digital imaging and communication in medicine (DICOM) (Figure 1(a)) [10]. In the second step, the 2D layer orientated DICOM format (Figure 1(b)) was reconstructed to a 3D-model [11]. For 3D-model-recovery, the image data was segmented with the 3D Slicer 2.6 program (www.slicer.org) to extract the bone volume area from the surrounding tissue and convert it to the STL format with the Visualization Toolkit 4.2 open source software [12]. On this CAD compatible basis, the geometry of the implant was designed according to the defect geometry by the CAD system in the third step by the CAD Pro/ENGINEER 3.0 software (ROTEC-Medizintechnik, Weisendorf, Germany) (Figure 1(c) and (d)).

In the fourth step, the implant geometry was exported from the CAD system in STL format which it is the most appropriate data format for rapid prototyping (Figure 1(e)) [13]. The next step was the data preparation for the selective laser sintering process itself; this included orientating, placing and slicing of the implant geometry in a virtual building chamber which was conducted with the RP-Tool 5.02 program (EOS, Krailing, Germany). This is a common process for near process data preparation and is done with system-related software which is capable of orientating, placing and slicing [13]. For the final step the implant was automatically fabricated layer by layer by a modified selective laser sintering system (EOSINT P 380, EOS, Krailing, Germany) (Figure 1(f)). Prior to implantation the specimen were sterilized using γ -irradiation sterilization which does not affect the mechanical properties of PEEK [14].

Surface Roughness and Scanning Electronic Microscopy

Scanning electronic microscopy (SEM) imaging of the implant surface was conducted with a XL30 Scanning Electron Microscope (Phillips, Eindhoven, Netherlands) at voltages ranging from 5 to 30 kV after the sample surfaces were gold sputtered (Pirani 501, Edwards, Crawly, UK) (Figure 2). The average surface roughness R_a of the three investigated groups was measured in accordance to the German Industry Norm (DIN)

Figure 1. Rapid prototyping of an implant for the reconstruction of the left zygomatic arch. The process from the CT until the fitted, individually shaped implant is demonstrated. (a) One slice of the computertomographical recording, (b) horizontal cross-section of the reconstructed 3D defect situation from bottom view with the missing zygomatic arch on the right side, (c) same view with the designed implant, (d) left side view of the skull with implant, (e) implant geometry as designed with CAD system, and (f) the laser sintered, (g) the defect situation of the skull model and (h) the reconstructed defect situation.

Figure 2. Scanning electronic microscopy pictures of the surface of the three different sample groups investigated in this study. (a) PEEK-CM, (b) PEEK-LS, and (c) PEEK-LS- β TCP (β -TCP is marked by an asterix).

4768 (μ -Surf Explorer, NanoFocus AG, Oberhausen, Germany). For PEEK-CM the average surface roughness was $0.32 \pm 0.04 \mu\text{m}$. The R_a value for the PEEK-LS was $5.01 \pm 1.12 \mu\text{m}$ and for the PEEK-LS- β TCP $7.53 \pm 0.61 \mu\text{m}$.

Surgical Implants

Since the rate of porcine bone regeneration (1.2–1.5 mm per day) is comparable to that of humans (1.0–1.5 mm per day) 10 adult domestic pigs were used in this study [15]. The protocol used was approved by the Committee for Animal Research, Franconia, Germany (Approval No 621-2531.31.16/05) utilizing a procedure that has been successfully used in previous studies [16,17].

All surgeries were performed using intubation anaesthesia. Streptomycin (0.5 g/d; Gruenenthal, Stolberg, Germany) was applied intramuscularly 1 hour pre-operatively and 2 days postoperatively. An incision was made to the skin and the periosteum of the skull to create access to the neurocranium. Nine identical critical size defects (CSD) (10 mm wide and 7 mm deep) per animal were created using a standardized trepan drill with a 10 mm diameter and 15 mm long (Ticom GmbH, Fürth, Germany) [16]. Three compression moulded PEEK implants (PEEK-CM), three laser sintered PEEK implants (PEEK-LS) and three laser sintered β -TCP containing PEEK implants (PEEK-LS- β TCP) were placed in the frontal skull of each pig. After placement of the implants the periosteum and skin were finally sutured in two layers (Vicryls 3.0; Vicryls 1.0; Ethicon GmbH & Co. KG, Norderstedt, Germany). The animals were sacrificed after 6, 12, and 24 weeks (3 animals at each point in time) using an intramuscular injection of azaperone (1 mg/kg) and midazolam (1 mg/kg) in the neck and euthanized by an intravascular injection of 20% pentobarbital solution until cardiac arrest occurred.

Histological Evaluations

The skull caps of the sacrificed animals were removed and immediately frozen at -80°C . The exact position of the implants was evaluated by X-ray (Faxitron Cabinet X-ray Systems, Illinois, USA) prior to explantation. Samples were immersed in 1.4% paraformaldehyde at 4°C in order to remove insoluble organic matrix constituents. Subsequently, the samples were dehydrated in an ascending alcohol series at room temperature in a dehydration unit (Shandon Citadel 1000, Shandon GmbH, Frankfurt, Germany). The explants were embedded using Technovit 9100s (Heraeus Kulzer, Kulzer Division, Werheim, Germany), which is suitable for the cutting and grinding technique suggested by Donath et al. [18]. The embedded bone specimens were reduced to $30\text{ }\mu\text{m}$, polished and the stirred continuously for 5 min in 10% H_2O_2 . They were then rinsed under cold running water, dried and stained in toluidine blue O solution for 15 min. Excess stain was removed by rinsing the specimens with distilled water.

Histomorphometrical Evaluations

For the histomorphometrical evaluations, the stained specimens was examined under a light microscope (Axio Imager.A1, Zeiss, Jena, Germany) equipped with a video camera (QICAM FAST 1394, Qimaging, Burnaby, Canada).

The measurements were performed along the outermost surface of the implants. The thickness of the surrounding fibrous tissue layers was evaluated at six different spots per implant (two at the bottom and two at both sides each).

Push-Out Test

After a 24-week implantation, the frontal skull of one pig was taken and the nine PEEK implants (three of each type) were tested for interfacial strength by a push-out test at room temperature using a tensile testing machine Zwick Z 050 (Zwick, Ulm, Germany). The bone plate with incorporated implants was grinded to get parallel surfaces. The specimen with the surrounding bone was placed on a metal support jig with a central circular opening with a diameter of 11 mm. The construction allowed an optical inspection from beneath by use of a mirror to ensure the central position of the specimen on the support jig. The force was applied by a plunger with a diameter of 9 mm. A preload of 0.2 N and a crosshead speed of 10 mm/min were applied. The shear strength (MPa)

of the polymer-bone interface was calculated by dividing the measured peak push-out load (N) by the interfacial area (π cylinder diameter [mm] bone plate thickness [mm]).

Statistical Analysis

Single-factor analysis of variance (ANOVA) for multiple comparisons was employed to assess the statistical significance of the data. A p -value of less than 0.05 was considered to be significant.

RESULTS

Histomorphometrical Evaluation

During the experimental period, all pigs remained in good health. At sacrifice, no macroscopic signs of inflammation or adverse tissue reaction were apparent at the implantation sites. Six weeks after implantation, all implants were surrounded by a connective tissue with a mild inflammatory reaction. The implant site contained a thin, fibrous layer. The mild inflammation reaction was characterized by the presence of polymorphonuclear leucocytes. The thickness of the connective tissue layer was $275.86 \pm 93.22 \mu\text{m}$ for the PEEK-CM group, $261.51 \pm 86.65 \mu\text{m}$ for the PEEK-LS group and $211.25 \pm 110.78 \mu\text{m}$ for the PEEK-LS- β TCP group. No statistically significance was measurable between the PEEK-CM and the PEEK-LS group ($p = 0.682$), the PEEK-CM and the PEEK-LS- β TCP group ($p = 0.121$) nor between the PEEK-LS and the PEEK-LS- β TCP group ($p = 0.199$) as seen in Figure 3.

After 12 weeks, there was no evidence of an inflammatory reaction, however, no direct bone implant contact was visible and all implants were encapsulated by fibrous tissue. The thickness of the capsule was $233.99 \pm 116.54 \mu\text{m}$ for the PEEK-CM group and $237.25 \pm 89.29 \mu\text{m}$ for the PEEK-LS group. The lowest value appeared for the PEEK-LS- β TCP group with $173.34 \pm 26.81 \mu\text{m}$. No statistically significant difference appeared between the PEEK-LS- β TCP and the PEEK-CM groups ($p = 0.191$), the PEEK-LS- β TCP and the PEEK-LS groups ($p = 0.332$) as well as between the PEEK-CM and the PEEK-LS groups ($p = 0.941$) (Figures 3 and 5).

The histomorphometrical evaluation of the implants 24 weeks after implantation showed a direct contact between superficially located β -TCP and the surrounding bone in the PEEK-LS- β TCP group (Figure 4). In contrast, no direct bone-implant contact was seen in the PEEK-CM and the PEEK-LS group. Both groups were surrounded by a thin fibrous

Figure 3. Thickness of the fibrous tissue isolating the implant from the surrounding bone. Even though the PEEK-LS- β TCP group shows the lowest values after 6, 12, and 24 weeks no statistically significant difference between the three different groups occurred throughout the study PEEK implant.

Figure 4. The interface between the implants and the surrounding bone of the three investigated groups 12 weeks after implantation. The presence of fibrous tissue isolating the implant from the surrounding bone is visible (a: PEEK-CM, b: PEEK-LS, c: PEEK-LS- β TCP, arrows indicate β -TCP granules enclosed in the implant).

layer without signs of an inflammatory reaction. The fibrous capsules surrounding the PEEK-CM and the PEEK-LS implants were $137.69 \pm 68.99 \mu\text{m}$ thick for the PEEK-CM g and $128.56 \pm 75.63 \mu\text{m}$ for the PEEK-LS groups, respectively. The thickness of the fibrous tissue

Figure 5. (a) Direct bone-implant contact between a PEEK-LS- β TCP implant and the surrounding bone 24 weeks after implantation. The bone is in direct contact with a superficially located β -TCP. (b) Direct bone-implant contact after an observation period of 24 weeks. Stars indicate β -TCP granular particles which are located on the surface. A thin fibrous layer is still visible between the implant surface and the surrounding bone (arrows).

that partly covered the PEEK-LS- β TCP implants was $88.13 \pm 51.16 \mu\text{m}$. No statistical difference was found between the PEEK-LS- β TCP and the PEEK-CM ($p = 0.059$) groups nor between the PEEK-LS- β TCP and the PEEK-LS groups ($p = 0.143$). Between the PEEK-LS and the PEEK-CM groups no statistical difference was found either ($p = 0.741$) (Figure 3).

During the 24-week period, no signs of degradation, breakage or mechanically instability of the PEEK implants were visible.

Push-Out Test

The performance of the PEEK implants after 24 weeks of implantation was characterized by a linear initial loading profile, followed by a well-defined peak load. The PEEK-LS- β TCP implants showed the highest interfacial strength within the three different groups with maximum shear strength of $0.53 \pm 0.08 \text{ MPa}$. In comparison the PEEK-CM and PEEK-LS implants had a significantly lower interfacial strength of $0.04 \pm 0.06 \text{ MPa}$ for PEEK-CM and $0.04 \pm 0.03 \text{ MPa}$ for the PEEK-LS implants. A statistical difference was found between the PEEK-LS- β TCP and the PEEK-CM implants ($p = 0.004$) as well as between the PEEK-LS- β TCP and the PEEK-LS samples ($p = 0.002$). However, no statistical differences appeared between the PEEK-CM and the PEEK-LS group ($p = 0.987$).

DISCUSSION

PEEK has excellent mechanical properties and bone-like stiffness and is considered to be an ideal alternative material for bone replacement while laser sintering is a potential process for building individually fitted implants in a short period of time. To demonstrate the practical application of the rapid-prototyping process used to generate the implants for individual trauma defect situations we developed an individually shaped laser sintered PEEK implant using computer tomography datasets (DICOM). The geometry of the defect was recorded and then segmented, reconstructed and formatted by appropriate software tools. Based on the software pre-processing and modification to the manufacturing process, it was possible to create a complex anatomical, individually fitted, laser sintered PEEK implant. The process is not limited to computer tomography datasets as implants can also be generated using datasets from other imaging techniques like Positron Emission Tomography (PET), 3D Ultra Sound Imaging (USI), Magnetic Resonance Imaging (MRI). This process may also benefit other surgical applications, such as trauma ear, nose and throat surgery.

The biocompatibility of PEEK is exceptionally interesting in that it exerts neither cytotoxicity nor mutagenicity *in vitro* [7,8,19]. However, cytotoxic effects on the surrounding tissue due to material properties of carbon black or the laser sintering process have not been evaluated *in vivo* yet. To evaluate the biocompatibility the critical size defect (CSD) was chosen; since this defect does not heal during a lifetime, it has been successfully used to evaluate bone substitution materials *in vivo* [16,20]. Our histopathological evaluation revealed that all of the PEEK implants were mainly surrounded by connective tissue and no signs of inflammatory response or necroses were visible. Using the definition of biocompatibility as the ability of a long-term implantable medical device to perform an intended function with the desired degree of incorporation by the host, without eliciting any undesirable local or systemic effects in that host [21] these implants can be characterized as bioinert [22]. Since the use of laser sintered PEEK and carbon black did not induce cytotoxicity under the study conditions, we consider these materials biocompatibility *in vivo* and suitable as long-term implant devices. Due to the current surgical complexity of reconstructing skull- and contour-defects in the face, this evaluation will be pursued in further animal studies.

The contact between superficially located β -TCP granules and the surrounding bone 24 weeks after implantation is an important find. A direct bone implant contact was possible since CaP ceramic surfaces are capable of conducting bone growth over the implant surface but are not able to induce bone formation *de novo* [23]. Therefore, the bone implant contact was due to the phenomenon of distance osteogenesis which also explains the formation of fibrous tissue on the implant surface. It has been reported that competition exists between fibroblasts and osteoblasts in order to populate the implant surface [24]. An essential observation of this phenomenon is that new bone does not form on the implant and that the implant will always be partially obscured from bone by intervening cells. Therefore, the initiation of mineralization of the healing bone tissue does not occur on the implant surface, but bone does grow towards the implant, subsequent to the death of the intervening tissue [25,26].

Besides a direct bone implant contact between surrounding bone and superficially located β -TCP granules, a resorption of the granules was visible 24 weeks after implantation and replaced by bone as described by Wiltfang and Merten [27]. This effect lead to a physical attachment of the implant in the bone with implant stability. The favorable effect of β -TCP on implant stability is also supported by the findings of the

push-out test. In comparison to the PEEK-CM and the PEEK-LS, the PEEK-LS-TCP showed significant higher fixation strength, whereas the other two groups were poorly fixated. This can be explained by the fibrous encapsulation of these implants, whereby no fixation strength can be achieved. No literature was found describing push-out tests for polymer implants, however, our results are in accordance to other studies using titanium or calcium phosphate implants [28,29]. To achieve a more secure attachment through a higher bone implant contact, a higher concentration of β -TCP could favor the interlocking between the implant and the surrounding bone. The problem is the location of the β -TCP in the specimens. On evaluating the implants, it was found that the β -TCP was located on the whole sample which means that only β -TCP granules or particles that are located on the surface effected bone formation, whereas the β -TCP trapped in the inner part of the implant were not effective. Therefore, locating the β -TCP granules on the outer surface would be desirable but not possible due to the current manufacturing process used. Unfortunately, a weight percentage of β -TCP higher than 10% disturbed the manufacturing process. Since a secure attachment between implant and bone did not occur, we added screw holes to the implant (Figure 1(f)) that enabled the surgeon to fixate a stable implant intra-operatively. It is very important to guarantee a correct facial contour without inflicting wound healing problems which may lead to the loss of the implant [30].

To achieve a higher interfacial strength, a pore architecture to influence the ingrowth of osteoblasts for a tight fit between implant and the surrounding tissue, is a possibility. A minimum pore size of 450 μm is needed to ensure the exploitation of a graft with blood vessels and to induce bone formation *in vivo* [31]. Although, we have achieved the prerequisite pore size with laser sinter porous PEEK-implants, the mechanical properties did not meet the requirements for reconstructive surgery implants [32].

Changes in the surface roughness could affect the osteointegration of the implants [33], however, no statistical difference in the results based on the thickness of the fibrous tissue layer between the different implants was observed. Therefore, surface roughness between 0.3 and 7.5 μm does not seem to affect the osteointegration of laser sintered and compression moulded PEEK in this type of experimental setup. A calcium phosphate coating used for plasma sprayed dental implants is another possibility. However, there are two main drawbacks for this coating; the thermal deterioration of the polymer composite due to the spraying process and the breakage of the coating [34].

CONCLUSION

This study is the first *in vivo* investigations of laser sintered PEEK implants with incorporated tricalciumphosphate as an osteoconductive composite. With the possibility to manufacture individually shaped, 3D biocompatible implants, laser sintered PEEK implants seem to be a viable innovative technique that would benefit patients and surgeons as a bone implant substitute for reconstructive surgery.

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