Processing of Three-Dimensional Laser Sintered Polyetheretherketone Composites and Testing of Osteoblast Proliferation in vitro

Dirk Pohle,[1] *Sabine Ponader,*[2] *Thomas Rechtenwald,*[3] *Michael Schmidt,*[3] *Karl Andreas Schlegel,*[2] *Helmut Münstedt,*[1] *Friedrich Wilhelm Neukam,*[2] *Emeka Nkenke,*[2] *Cornelius von Wilmowsky**[2]

Summary: The following investigation focuses on polyetheretherketone (PEEK) for bone substitutes intended for maxillofacial surgery. Different three-dimensional discs with a diameter of 12 mm and a height of 3 mm were laser sintered. As filler materials nano-sized carbon black and β-tricalciumphosphate powder with an average grain size of 35 μm were used. Human osteoblasts were cultivated on the discs and examined with scanning electron microscopy. Cell vitality and cell growth was investigated. The data shows that PEEK surfaces does not suppress osteoblast proliferation.

Keywords: high performance polymers; human osteoblast; In vitro proliferation; laser sintering (LS); polyetheretherketone (PEEK)

Introduction

Polyetheretherketone (PEEK) is a high performance, semi-crystalline thermoplastic polymer. It combines a very good strength and stiffness with an excellent thermal and chemical resistance against oils, acids and biological fluids. Its continuous use temperature is 260 °C. Hence the polymer is suitable for every clinical sterilisation method. Due to its excellent biocompatibility PEEK is routinely used in long-term medical implant applications.[1] These parts are typically produced by conventional manufacturing methods like injection moulding. Especially the production of individually shaped implants would benefit from a more flexible manufacturing technique.

With respect to the direct production of individually shaped implants laser sintering (LS) can be seen as an ideal production technology. A schematic drawing of a LS-equipment is shown in Figure 1. LS enables the direct manufacturing of products with complex geometries, including undercuts and defined porosity.[2,3] Although its productivity can not compete with mass production technologies, as the LS manufacturing process needs several hours, it is convenient for direct fabrication of single parts with an individual shape, such as medical implants.

Similar to most rapid prototyping processes the directly produced specimen is based upon digital computer data, for example computer tomography. The material is heated above its melting temperature by a CO_2 laser, following the data of the CAD model. The viscous particles form sintering necks within the top powder layer. After finishing one layer and lowering the building platform a new powder layer is applied on top of the previous one and the

[1] Institute of Polymer Materials, Department of Materials Science, University of Erlangen-Nuremberg, Erlangen, Germany
[2] Department of Oral and Maxillofacial Surgery, University of Erlangen-Nuremberg, Erlangen, Germany
Fax: 0049 (0)9131 85 36288
E-mail: Cornelius.von-Wilmowsky@mkg.imed.uni-erlangen.de
[3] Bavarian Laser Center gGmbH, Erlangen, Germany

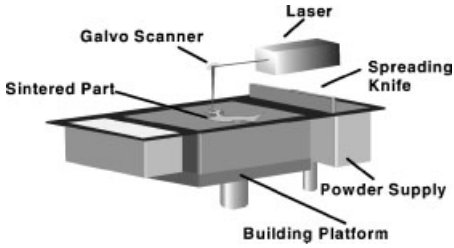

Figure 1.
Principle of laser sintering (LS).

laser treatment process starts again. Thus, a three-dimensional specimen can be generated layer by layer.

The aim of this study is to show that laser sintered PEEK is suitable for the use of implant material because of its potential for individually shaped implants with good mechanical properties. Samples were tested for cell vitality and proliferation. Furthermore, it was investigated if it is possible to add inorganic, non-melting materials into laser sintered PEEK samples. Therefore β-TCP was chosen, because of its routinely clinical use as bone substitution material in various forms.[4] Effects on osteoblasts in vitro were investigated.

Materials and Methods

PEEK Powder Processing

For the purpose of this research the powder PEEK 150 PF (Victrex Plc., Lancashire, UK), with a low melt viscosity and an average particle size of 50 μm was used (Figure 2). This powder has a broad grain size distribution from 5 μm to 110 μm, measured by laser diffraction (Mastersizer 2000, Malvern Instrument Ltd, UK).

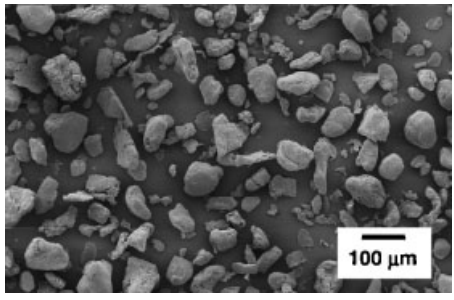

Figure 2.
SEM-picture of the commercial powder PEEK 150 PF.

For the laser sintering process, with layer thicknesses of 100 μm, it was necessary to sieve the powder below 63 μm using a sieve shaker (AS 200, Retsch GmbH, Germany). Commercial PEEK powder exhibits irregular, edged particles. Due to particle geometry and its broad grain size distribution, the application of the powder is strongly disturbed, as it does not flow smoothly. By adding 1 wt% of carbon black, powder flow ability is increased. This modification enables an automated process for the application of a thin, homogenous powder layer. Repose angle measurements demonstrated this positive effect. For pure PEEK 150PF powder a repose angle of 41° was measured, by adding 1 wt% of carbon black the repose angle decreased to 27°. Nano-sized carbon black was delivered by Degussa AG (Frankfurt, Germany). In addition to carbon black the PEEK powder was compounded with 10 wt% β-tricalcium phosphate (β–TCP, Merck, Darmstadt, Germany), to examine the possibility of adding inorganic materials to the polymer powder in the LS process. The β-TCP was chosen because of its clinical appliance in various forms as a bone substitution material.[4] The β-TCP powder used was classified as dry and purest, following European pharmaceutical guidelines (E141, Ph Eur, British Pharmacy). 98% of the particles have a smaller diameter than 63 μm as stated by the manufacturer.

Laser Sintering Machine Modification

To generate three dimensional PEEK specimens for in vitro testing it was necessary to establish a furnace temperature of 340 °C throughout the whole process. Standard laser sintering machines do not fulfil this requirement. Instead of building a completely new machinery in order to solve this problem the EOSINT P 380 (EOS, Krailing, Germany) sintering system was modified. The most critical point in this context is the subsystem, necessary for preheating the newly applied powder layers. Protection of all temperature sensitive

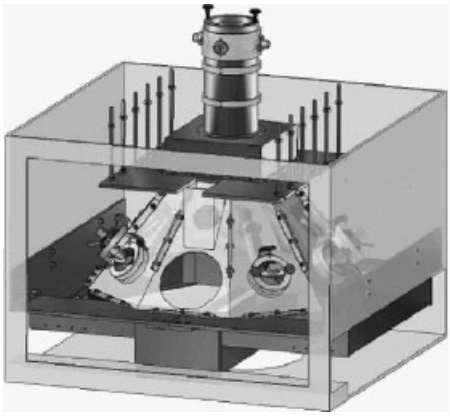

Figure 3.
Sketch of the "Heating dome", which provides preheating of polymer powder up to 345 °C in a standard laser sintering machine.

machine elements like the optical lens (T_{max}: 150 °C) or stepper sensors (T_{max}: 70 °C) has to be ensured.

Due to the high melting temperature of PEEK (345 °C) the temperature value for preheating the polymer powder has to be unusually high for polymer processing. Preheating has to be carried out very uniformly over the complete building platform and the process has to be performed within a small tolerance range.

The ability for an automated and properly controlled generation of complex shaped prototypes is an additional objective of the work. Due to these requirements and constraints a new concept of a scaled-down, open and high-temperature inner process chamber called "Heating Dome" had to be developed. Figure 3 shows a scheme of the realised modification.

Samples

Specimens tested for osteoblast vitality and proliferation were pure PEEK, PEEK with 1 wt% carbon black, PEEK with 1 wt% carbon black and 10 wt% β-TCP. The laser sintered discs used for the proliferation tests had a diameter of 12 mm and a height of 3 mm. Osteoblasts seeded on tissue culture plates were used as control.

Cell Culture

Human foetal osteoblasts (hFOB 1.19) were seeded in T-150 culture flasks with fresh culture media (DMEM/F12, Invitrogen Life Technologies, Germany) supplemented with 10% fetal calf serum (Gibco BRL, Paisley, Scotland, United Kingdom), 105 I.U. penicillin and 100 mg/l streptomycin (Abbod GmbH, Germany). Ten thousand cells/cm^2 were added to the surface of each biomaterial in a well of tissue culture polystyrene (TCPS). Cells were incubated at 34 °C under humidified 5% CO_2 conditions.

Cell Proliferation Assay

Cell proliferation was assessed using the labelling kit WST-1 (ROCHE Diagnostics GmbH, Mannheim, Germany) after 1, 3, 5, 7 and 14 days. The assay was performed according to the manufacturer's instructions. Culturing medium was removed and 450 µl of fresh medium and 50 µl WST-1 labelling solution was added to each well. The determination of cell proliferation was performed after 2 h in a micro plate ELISA-reader (Vmax, Molecular Devices, Germany) at an absorbance of 450 nm.

Analysis of Cell Vitality

A combined staining of fluorescein diacetat (FDA) (5 mg/ml in acetone, Sigma, Germany) and propidium iodide (PI) (1 mg/ml in distilled water, Sigma, Germany) was used to investigate cell vitality. The samples were carefully covered with that dye. After an incubation of one minute the solution was removed. Samples were observed with an inverse microscope (Axioskop, Zeiss, Germany). Cell vitality was quantified by counting the number of living and dead cells for each sample at five different spots with a 10×/0.3 (Plan-Neofluar, Zeiss, Germany) magnification.

Statistical Analysis

All measurements were performed at least five times and expressed as the mean. 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

By pre-processing of the powder and with regard to preheating temperature three-dimensional laser sintered parts, suitable for cell testing in vitro were obtained (Figure 4).

After 24 hours in culture cell vitality was investigated by FDA/PI staining and the ratio of living to dead cells was evaluated (Table 1). A cell vitality of 98.2% could be found for the control group (hFOB 1.19), 95.3% for pure PEEK and 96.4% for PEEK with 1 wt% carbon. Samples were homogenously covered with well spread cells (Figure 5a and b). On PEEK scaffolds with 1 wt% carbon black and 10 wt% β-TCP a cell vitality of 83.2% was found with a reduced cell density. Some of the visualized cells were rounded and cell spreading was limited (Figure 5c). All of the groups showed cellular proliferation during 14 days of culturing indicating that PEEK does not induce cytotoxicity under these conditions. After 14 days the highest proliferation was obtained for the control group and lowest proliferation rate occurred for PEEK containing 10 wt% β-TCP (Figure 6). Results showed a significantly reduced proliferation for PEEK/1 wt% carbon black ($p = 0.0049$) between day 1 and 3 and for PEEK/1 wt% carbon black/10 wt% β-TCP ($p = 0.005$).

Table 1.
Cell vitality of different laser sintered PEEK scaffolds.

Sample	Vitality (%)
PEEK pure	95.3
PEEK 1 wt% carbon black	96.4
PEEK 1 wt% carbon black, 10 wt% ß-TCP	83.2
HFOB 1.19	98.2

The same effect was observed for PEEK/1 wt% carbon /10 wt% β-TCP between day 5 and 7. A permanent growth rate of both samples between day 7 and 14 could be proved, suggesting that carbon black or β-TCP might have a negative effect during the early stage of proliferation, only. A slightly reduced proliferation of osteoblasts on the pure PEEK samples between day 1 and 3 was not significant ($p = 0.6884$). Constant proliferation occurred for the control group (Figure 6).

Discussion

In the present study the cell vitality and proliferation was investigated over 14 days. According to other studies in the literature on the biocompatibility of PEEK,[5] the results of the present study show that laser sintered PEEK allows cell proliferation and does not induce cytotoxicity under chosen study conditions. A significant higher proliferation of PEEK/1 wt% carbon black compared to pure PEEK ($p = 0.0084$) at day 14 indicates that the addition of carbon black for the reason of better manufacturing conditions has no cytotoxic effect on

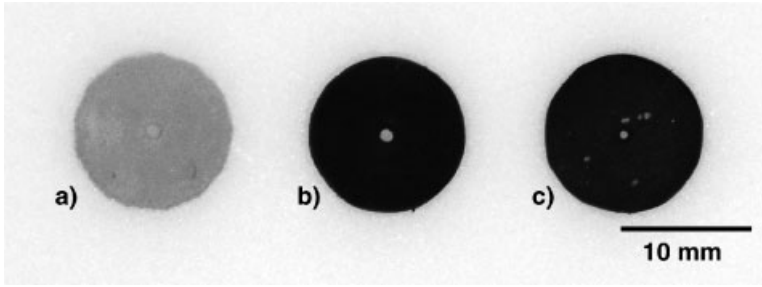

Figure 4.
Photograph of different cell test specimens a) pure PEEK b) PEEK with 1 wt% carbon black c) PEEK with 1 wt% carbon black and 10 wt% of ß-TCP.

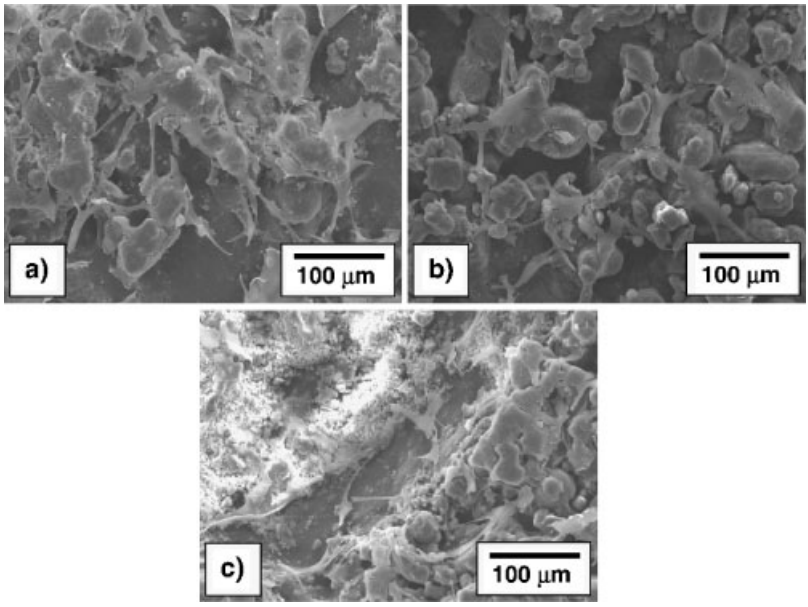

Figure 5.
SEM-Picture of sample surfaces with human osteoblasts a) pure PEEK b) PEEK with 1 wt% carbon black c) PEEK with 1 wt% carbon black and 10 wt% of ß-TCP.

cellular growth but might inhibit the early stage of osteoblast proliferation in vitro. It could be demonstrated that the proliferation rate is reduced on samples added with β-TCP. This may lead to the conclusion that calcium phosphate compounds at a concentration of 10 wt% might have no favourable effect on osteoblast proliferation in vitro. The finding that proliferation was reduced, but not inhibited, suggests that β-TCP-PEEK does not induce cytotoxicity but reduces cell proliferation under these study conditions. This finding matches with the results of previous studies, showing that β-TCP has an inhibitory effect on osteoblasts growth in vitro.[6,7,8]

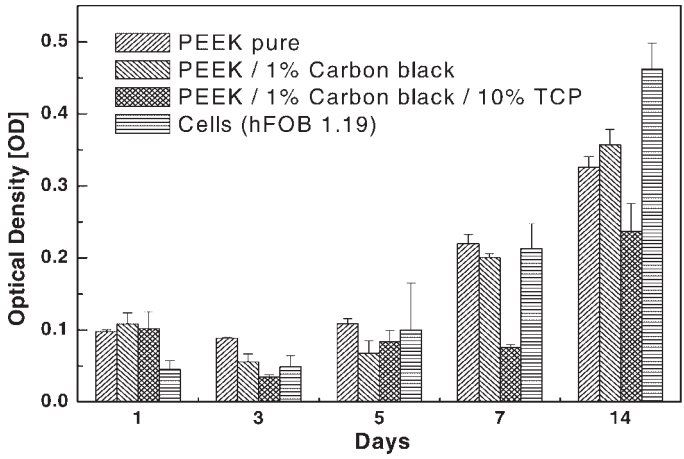

Figure 6.
Proliferation of osteoblast cells.

Conclusion

Due to laser sintering machine modification and pre-treatment of polymer powder it was possible to manufacture three-dimensional parts made of PEEK by laser sintering. Cell test specimens consisting of pure PEEK, PEEK/1 wt% carbon and PEEK/1 wt% carbon /10 wt% β-TCP were generated. Cell proliferation tests have shown that cytotoxicity is not induced on laser sintered PEEK and PEEK samples containing carbon black, but showed no advantageous effect of PEEK composites containing 10 wt% of calcium phosphate on the proliferation of osteoblasts in vitro. Laser sintered PEEK parts seem to be attractive candidates for the use as possible bone analogue substitute due to their biocompatibility, individual shape and the possibility of compounding bioinert polymer powder with other materials which might benefit bone formation in vivo.

[1] J. Jahur-Grodzinski, Review - Biomedical application of functional polymers. In: *Reactive and Functional Polymers* **1999**, *39*, 99–138.
[2] J. P. Kruth, Material Incress Manufacturing by Rapid Prototyping Techniques. In: *CIRP Annals* **1991**, *40:2*, 603–614.
[3] K. K. B. Hon, T. J. Gill, Selective Laser Sintering of SiC/Polyamide Composites. In: *CIRP Annals* **2003**, *52:1*, 173–176.
[4] M. Bohner, Calcium orthophosphates in medicine: from ceramics to calcium phosphate cements. *Injury* **2000**, *31* (Suppl 4), 37–47.
[5] L. Petrovic, D. Pohle, H. Münstedt, T. Rechtenwald, K. A. Schlegel, S. Rupprecht, Effect of βTCP filled polyetheretherketone on osteoblast cell proliferation in vitro. *J. Biomed. Science* **2006**, *13*, 41–46.
[6] D. Williams, New horizons for thermoplastic polymers. *Med. Device Technol.* **2001**, *12*, 8–9.
[7] A. Katzer, H. Marquardt, J. Westendorf, J. V. Wening, G. von Foerster, Polyetheretherketone-cytotoxicity and mutagenicity in vitro. *Biomaterials* **2002**, *23*, 1749–1759.
[8] J. S. Sun, Y. H. Tsuang, C. J. Liao, H. C. Liu, Y. S. Hang, F. H. Lin, The effects of calcium phosphate particles on the growth of osteoblasts. *J. Biomed. Mater. Res.* **1997**, *37*, 324–334.

