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Plasma treatment on novel carbon fiber reinforced PEEK cages to enhance bioactivity

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Abstract: Carbon fiber reinforced polyetheretherketone (CFR-PEEK) has similar mechanical properties to human bone and is considered as the best alternative material to substitute titanium for spine cage implants. To compensate its poor osteogenic properties and limited bioinertness, CFR-PEEK was coated with a thin film of titanium. In the study, we investigated the biological response *in vitro* of titanium coated CFR-PEEK with different vacuum plasma pretreatments. The so modified surface revealed first hints for a good cell response by excellent cell adhesion and morphology of human osteoblast – like cells MG 63 (ATXX:'CRL-1427). Thus, the findings show that surface roughness of CFR-PEEK material has a profound effect on the biological activity via vacuum plasma treatment.

Keywords: cell tests; CFR PEEK; osseointegration; plasma; PVD; spinal cages; surface roughness.

1 Introduction

Up to 80% of the population suffer dorsal pain at some time during life. Most of back problems get better without a surgical procedure. There are many different causes of back pain and various types of treatments. In case of posttraumatic stress disorder i.e. spine instability or degenerative disk disease, defined as abnormal movement between one vertebrae to another, it cannot be always cured by conventional therapy. Thus, a lumbar spinal fusion surgery is performed to stop the motion of the painful vertebral segment and to strengthen the vertebral body. One approach is to remove the disk and vertebral bodies and replace a vertebrae with spinal cage implant (Figure 1).

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The requirements for those implants are biocompatibility, cell adhesion, sufficient biomechanical behavior (e.g. modulus of elasticity) and osteoconductivity. Titanium is the material mostly used for such implants [1]. However, titanium has disadvantages for this application. Its stiffness, which is ten times higher than human bones, can lead to unbalances between the implant and spine and results in loosening of the implants due to stress shielding effect. Another disadvantage is the metal-induced artefact in computed tomography (CT) and magnetic resonance imaging (MRI) that consequently reduces drastically the ingrowth control of the implant. To overcome those drawbacks, polyetheretherketone (PEEK) is becoming increasingly popular as an alternative material for implants. This thermoplastic polymer possesses excellent biocompatibility, ductility, elongation and fatigue resistance, and its elastic modulus is close to human bones. Furthermore, PEEK does not induce image artefacts and allows a better control of implant using MRI [2, 3]. However, deficient osteogenic properties limits its fields of application. PEEK is not used in the lumbar spine region due to the extreme high loads in this area, which may cause e.g. pseudarthrosis and loosening of the implant. Carbon fiber reinforced (CFR) PEEK is a possible material to overcome the mechanical weakness. Added carbon fibers improve the mechanical resistance and ductility compared to pure PEEK material. Surface modification of CFR PEEK promotes the osteoblast attachment and growth. A functional titanium coating is applied and offers the best possible osseointegration of CFR PEEK implants.

This study assesses the *in vitro* osseointegration of CFR PEEK implants coated with titanium. Before coating, various vacuum plasma treatments were carried out. The aim is to evaluate the effect of surface properties i.e. surface roughness to reduce the osseointegration period for the proposed spinal cage implant [4].

2 Methods

Different surface treatments *in vacuo* were performed before titanium deposition. Cylindrically formed CFR

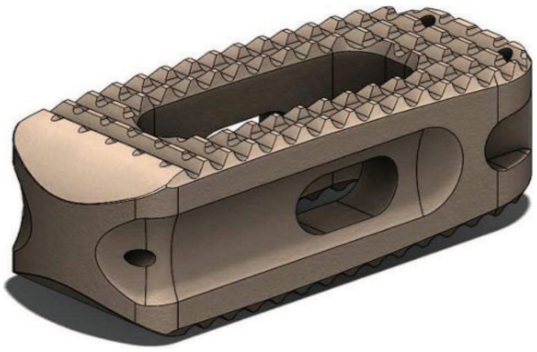

Figure 1: Cage for posterior lumbal interbody fusion PLIF (Silony Medical GmbH, Germany).

PEEK substrates were specially prepared for this study with a diameter of 30 mm and a thickness of 4 mm. For cell tests the cylindrical substrates were cut into quarters. The CFR PEEK substrates were provided by Silony Medical GmbH, Germany.

2.1 Surface roughness analysis

To characterize the roughness before and after surface treatments a roughness tester PCR-RT 1200 (PCE Instruments, Germany) was used. The roughness tester works according to the piezoelectric micro probe principle like the highly accurate laboratory measuring instrument. The roughness tester scans the surface within seconds and shows digitally either the value R_a, R_z, R_q or R_t.

2.2 Face milling

The CFR PEEK substrates were milled to get a suitable surface roughness before plasma treatment and deposition steps. The milling process was done with a DECKEL FP1 machine (Friedrich Deckel AG, Germany). Following milling parameter were set to reach a common surface roughness of $R_a = 0.9$ µm.

The milling speed applied was 600 rotation per min, feed rate 50 mm/min, number of teeth 6, and diameter 40 mm.

2.3 Plasma treatment and coating

Plasma treatment and the titanium deposition were done with a combined Physical Vapor Deposition and Plasma Enhanced Chemical Vapor Deposition (PVD&PECVD) machine Porta 350 (Plasma Electronic GmbH, Germany). In this study, we performed two different plasma treatments on CFR PEEK substrates before titanium deposition. Samples were placed in the vacuum chamber and fixed on a sample holder connected to a radio frequency (RF) generator and pumped down to 2×10^{-3} Pa. The first plasma treatment was performed using an argon plasma with 80 sccm gas flow at 0.37 Pa. As alternative plasma treatment, we carried out an argon and oxygen gas mixture at 0.51 Pa with 70 sccm and 30 sccm, respectively. Substrates were treated for 3 h at 330 W and 350 V bias. After plasma treatment, the substrates were coated in the same vacuum chamber without breaking the vacuum.

The deposition method of the titanium films was unbalanced magnetron direct current (DC) sputtering. The background pressure was maintained at 2×10^{-3} Pa. Titanium growth was deposited with an argon flow of 30 sccm and at a pressure of 0.11 Pa during 30 min at 2000 W DC power and target voltage of 410 V. A 240 nm thin titanium layer was deposited on treated and untreated CFR-PEEK samples. Two different bias voltages were applied 0 and 200 V during titanium deposition.

2.4 Coating adhesion

The film adhesion was quantified by cross-cut tests in accordance with standard DIN EN ISO 2409:2007. It describes a test method for assessing the resistance of coatings to separation from substrates when a right-angle lattice pattern is cut into the coating, penetrating through to the substrate. Then coating adhesion was measured by tape test method. A specific adhesive tape was applied to the pattern and pulled off at constant speed at an angle of approximately 60 degrees.

2.5 Cell adhesion & morphology

In order to collect first hints for a good tissue integration of the modified CFR PEEK implant, cell adhesion and morphology of human osteoblast-like MG-63 P91 cells, were seeded at different treated CFR PEEK substrates.

After sterilization of the substrates by incubation in 70% Ethanol for > 1 h they were placed in a cell culture 12 well plate and seeded with MG-63 cells with a cell density of 20,000 cells/cm^2. The duration of cell adhesion was 20 h at 37°C and 5% CO_2 under static conditions. As culture medium Dulbecco's Modified Eagle Medium (DMEM; high glucose) supplemented with 10% fetal calf serum, 10,000 U/ml penicillin and 10,000 µg/ml streptomycin was used.

After cell seeding an actin staining (= cytoskelet-staining) with phalloidin oregon green (Thermo Fisher Scientific) was performed to monitor cell morphology of the adhered osteoblast-like cells. Between all staining steps substrates were washed at least once with phosphate buffered saline (PBS). All steps were realized at room temperature. To fix the cells substrates were incubated with 4% paraformaldehyde/PBS for 30 min. Since actin is a part of the cytoskelet cells were permeabilized with 0.02% TitonX100/dH_2O for 15 min to get access to the antigen. For blocking unspecific binding of the phalloidin the fixed and permeabilized cells were treated with a solution of 1% bovine serum albumin/PBS for 45 min. Finally samples were stained with phalloidin oregon green/PBS solution (Dilution: 1:50) for 3 h. In order to stain cell nuclei additionally a second staining with 5 µg/ml 4′,6-Diamidin-2-phenylindol (DAPI)/PBS for 10 min was performed.

To evaluate the adhesion-rate and morphology of the adhered cells, the so prepared substrates were investigated by fluorescence microscopy.

3 Results and discussion

Milling tests showed, that carbon reinforced PEEK is machinable with standard milling processes. After the milling step, we measured surface roughness (R_a) of substrates between 0.5 µm to 1 µm. The milling process creates groove shapes on the substrate surface. These undesirable grooves are responsible for the inhomogeneous surface roughness. It has been determined that standard machining process reached his limits and it is not possible to treat undercuts, contrary to plasma processes. With an argon or argon/oxygen plasma treatment it is possible to adjust a perfect and homogeneous surface roughness, even in undercuts. After 3 h plasma treatment, surface roughness (R_a) between 1.5 µm and 1.8 µm are achieved. Samples 1–9 were treated with Ar/O_2 plasma. Samples 1–5 and 6–9 were treated with a generator power of 500 W and 750 W, so plasma treatment increases the roughness up to 100% (Figure 2).

After argon and oxygen mixed plasma treatment, samples showed no adhesion of the titanium layer to the CFR PEEK for 0 and 200 V bias, as highlighted in Figure 3-left. This might be due to strong and irreversible surface reactions between PEEK and oxygen plasma.

Substrates treated under argon plasma and coated with 0 V bias showed very good adhesion according to DIN EN ISO 2409:2007. After tape test, no delamination was observed, as shown in Figure 4.

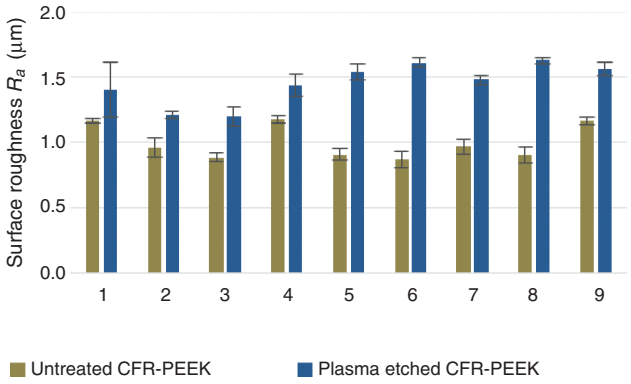

Figure 2: Roughness before and after plasma treatment. Sample 1–5 were etched by 500 V; Sample 5–9 at 750 V.

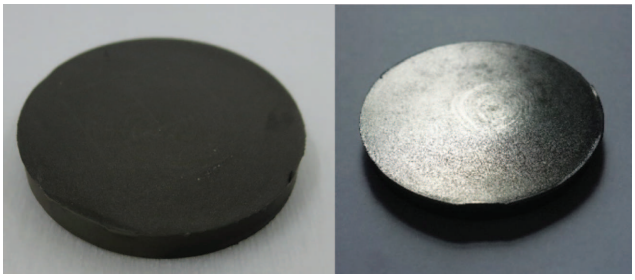

Figure 3: Substrate treated with Ar/O_2 plasma and 200 V bias during titanium deposition (left) and treated with pure argon plasma and titanium coated (right) CFR PEEK.

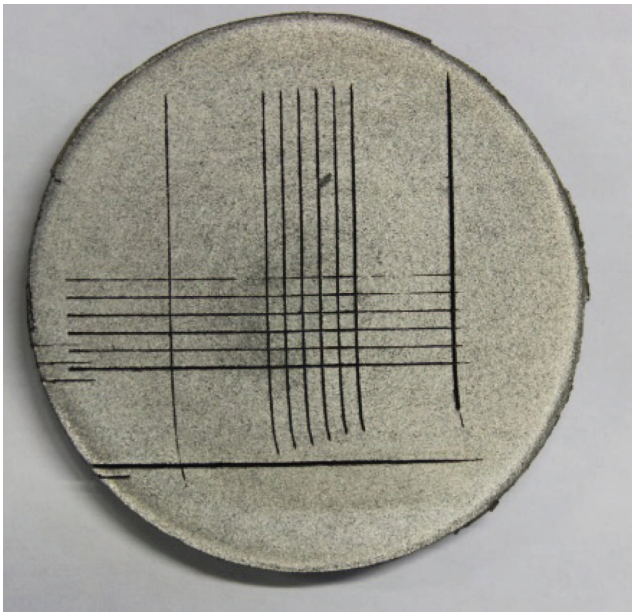

Figure 4: Titanium coated substrate after cross cutting test.

The results showed, that low bias voltage at 0 V yield better layer adhesions. However high bias voltage at 200 V increase drastically the surface roughness. However,

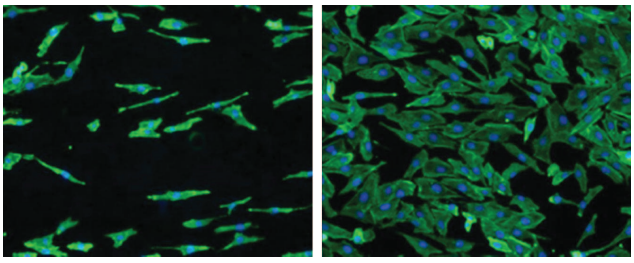

Figure 5: Fluorescence microscopy pictures of cell adhesion and morphology of MG-63 cells on different titan-coated CFR-PEEK substrates (left: without plasma treatment; right: with plasma treatment. Green: actin, stained with phalloidin Oregon green, Blue: cell nuclei stained with DAPI. The dark areas correspond with the milling grooves.

the influence of bias voltages during deposition needs to be clarified.

After titanium deposition onto CFR-PEEK substrates, cell tests were carried out to evaluate the adhesion and morphology of seeded MG-63 osteoblastic-like cells. For this test, two different substrates and one standard cell culture plastic as positive control were populated with cells. One substrate without milling and plasma treatment, another substrate milled and plasma treated with a surface roughness of $R_a = 1.9$ µm after treatment. The results showed that cells adhere on the plasma treated and coated CFR-PEEK substrates.

MG-63 cells showed a comparable adhesion-rate to the positive control and a typical well spread morphology of viable MG-63 cells, as depicted in Figure 5 on right side. In Figure 5 (left side), the untreated substrate showed reduced adhesion rate of about 30% compared to the plasma treated CFR-PEEK sample and the cell culture reference. Furthermore, cells settled down on untreated substrate showed a narrow cell morphology due to preferable orientation on the milling grooves.

4 Summary

In this study, we showed that a homogeneous surface roughness of CFR-PEEK can be tuned using argon plasma treatment. It was also shown that adhesive thin titanium films could be deposited on CFR PEEK using pure argon plasma treatment.

Biological activity of titanium coated CFR-PEEK substrates was proven with human osteoblastic-like cells MG-63. It was demonstrated that cells adhere well on the treated and coated implant surface. The cells proliferate very well on surfaces with a roughness around 2 µm. The optimal surface roughness and layer thickness have to be investigated in further tests. It is also necessary to conduct toxicity tests at the optimized CFR PEEK surfaces.

Implants of this kind are massively loaded mechanically while implantation. So it is also important to test the layer adhesion under high loads. Tensile and peel tests can be carried out to characterize the layer adhesion.

Author's Statement

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