Aus dem Institut für Zahn-, Mund- und Kieferheilkunde
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

The impact of different low-pressure plasma types on the physical, chemical and biological surface properties of PEEK

Der Einfluss unterschiedlicher Niederdruckplasmabehandlungen auf die physikalischen, chemischen und biologischen Oberflächeneigenschaften von PEEK

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List of abbreviations

Ar	*argon*
AC	*acrylic acid*
AA	*allylamine*
CI [%]	*crystallinity index*
FDA	*fluorescein diacetate*
FTIR	*fourier-transform infrared spectroscopy*
H	*hydrogen*
HOB	*human osteoblasts*
LPPT	*low-pressure plasma treatment*
O	*oxygen*
PEEK	*poly-ether-ether-ketone*
PIII	*plasma immersion ion implantation*
Ra	*surface roughness*

Abstract

Due to its outstanding material properties, polyether ether ketone (PEEK) is increasingly used as a medical device material. But its surface energy is too low e.g. to achieve sufficient osseointegration, resulting in a biologically inert surface. Plasma treatment can modify the surface properties of PEEK by improving the surface energy and biological activity of PEEK and thus its osseointegration behavior. Plasma treatment can also improve the bonding strength between PEEK denture frameworks and veneering composites. The changes of PEEK surface characteristics after low-pressure plasma treatment (LPPT) may be based on both, physical and chemical changes. A better understanding of the effects induced by plasma treatment seems to be useful since low-pressure plasma processses show a high reproducibility. By now, there is no specific research reporting on the effects of LPPT on various surface characteristics of PEEK. Therefore, the purpose of this study was to assess the roughness, hydrophilicity, microhardness, crystallinity and biological activity of PEEK surfaces after the exposure to different LPPTs.

For this, 218 PEEK samples in the shape of round discs were prepared and divided into 4 groups, according to the different LPPTs:

1. Untreated PEEK (n=32, without LPPT)

2. H-PEEK (n=62, hydrogen LPPT)

3. O-PEEK (n=62, oxygen LPPT)

4. H/O-PEEK (n=62, hydrogen/oxygen LPPT, mixing ratio 2:1)

Compared to untreated PEEK, the hydrophilicity, surface crystallinity and micro-hardness of PEEK after LPPT were significantly increased, whereas the O-PEEK and the H/O-PEEK groups showed the highest hydrophilicity with a contact angle close to 0 degrees. This property occurred ten times faster under hydrogen/oxygen LPPT than under oxygen LPPT. Using a test force of 0.02 N, all groups showed significantly different micro-hardnesses.

Cell culture tests with human osteoblasts (HOB) revealed significantly higher cell densities on plasma-treated PEEK surfaces compared to untreated PEEK, which might be due to better cell adhesion.

The changes may have been caused by both the size and the chemical properties of the specific atoms used in the plasma chamber. In the future, further tests should be conducted to

assess the duration of LPPT that causes the highest crystallinity. The effects on osseointegration should also be evaluated in vivo.

Zusammenfassung

Aufgrund seiner herausragenden Materialeigenschaften wird Polyetheretherketon (PEEK) zunehmend als Medizinproduktmaterial eingesetzt. Doch seine Oberflächenenergie ist z. B. für eine ausreichende Osseointegration zu gering, was zu einer biologisch inerten Oberfläche führt. Eine Plasmabehandlung kann die Oberflächeneigenschaften von PEEK verändern, indem sie die Oberflächenenergie und die biologische Aktivität von PEEK und damit das Osseointegrationsverhalten sowie die Haftfestigkeit zwischen Verblendkompositmaterialien und PEEK-Prothesengerüsten verbessert. Diese Veränderungen der Oberflächeneigenschaften können auf physikalischen und chemischen Veränderungen der PEEK-Oberfläche beruhen. Für ein besseres Verständnis der durch die Plasmabehandlung induzierten Effekte könnte das Niederdruck-Plasmaverfahren aufgrund seiner hohen Reproduzierbarkeit hilfreich sein. Bis jetzt gibt es keine spezifischen Untersuchungen, die über die Auswirkungen der Niederdruck-Plasmabehandlung auf verschiedene Oberflächeneigenschaften von PEEK berichten. Daher war das Ziel dieser Studie, die Rauheit, Hydrophilie, Mikrohärte, Kristallinität und biologische Aktivität von PEEK-Oberflächen nach verschiedenen Niederdruck-Plasmabehandlungen zu bewerten.

Dazu wurden 218 PEEK-Proben in Form von runden Scheiben hergestellt und entsprechend der verschiedenen Plasma-Oberflächenbehandlungen in 4 Gruppen eingeteilt:

1. Unbehandeltes PEEK (n=32, ohne Plasmabehandlung)

2. H-PEEK (n=62, Wasserstoff-Plasmabehandlung)

3. O-PEEK (n=62, Sauerstoff-Plasmabehandlung)

4. H/O-PEEK (n=62, Wasserstoff/Sauerstoff-Plasmabehandlung, Mischungsverhältnis 2:1)

Im Vergleich zu unbehandeltem PEEK waren die Hydrophilie, Oberflächenkristallinität und Mikrohärte der PEEK-Oberflächen nach den Plasmabehandlungen signifikant erhöht, wobei die O-PEEK-Gruppe und die H/O-PEEK-Gruppe die höchste Hydrophilie mit einem Kontaktwinkel nahe 0 Grad aufwiesen. Dieser Effekt stellate sich in der H/O-PEEK-Gruppe 10-mal schneller ein als in der O-PEEK-Gruppe.

Bei Verwendung einer Prüfkraft von 0,02 N zeigten alle Gruppen signifikant unterschiedliche Mikrohärten.

Zellkulturtests mit humanen Osteoblasten (HOB) zeigten signifikant höhere Zelldichten auf plasmabehandelten PEEK-Oberflächen im Vergleich zu unbehandeltem PEEK, was auf eine verbesserte Zelladhäsion zurückgeführt werden könnte.

Die Veränderungen können sowohl durch die Größe als auch durch die chemischen Eigenschaften der spezifischen Atome, die in der Plasmakammer verwendet wurden, verursacht worden sein. In Zukunft sollten weitere Tests durchgeführt werden, um die maximale Plasmabehandlungszeit zu ermitteln, die zu einer maximalen Kristallinität führt. Die Auswirkungen auf die Osseointegration sollten auch in vivo evaluiert werden.

1. Introduction

A complete dentition has a significant positive impact on the oral health-related quality of life (OHRQoL), whereas tooth loss causes the loss of different tissues forming the orofacial anatomy in the long-term, including bone tissue, nerves and muscles negatively affecting orofacial functions such as chewing ability. Negative effects on OHRQoL may also derive from language barriers, pain, and the lack of self-satisfaction with regard to one's own appearance [1]. However, due to dental caries, trauma, periodontal disease and other reasons, many people will face the problem of tooth loss. According to the German oral health studies survey, in 2014, the average population aged 65 to 74 lacked 11,1 teeth per person, and the average population aged 35 to 44 lacked 2.1 teeth per person [2]. It is estimated that in 2030, the average population aged 65 to 74 will lack 6.75 teeth per person, and the average population aged 35 to 44 will lack 1.2 teeth per person [2].

At present, there are different kinds of restoration used to replace missing teeth, such as complete dentures, removable partial dentures, fixed fixed partial dentures supported by teeth and/or dental implants. A reduced amount of teeth and wearing a complete denture reduces the efficiency of the chewing ability significantly, what could affect the general health status of these patients [1]. Therefore, the use of dental implants to anchor dental restorations has a positive effect on the quality of life [3]. Moreover, to avoid shaping and grinding and thus damaging the adjacent teeth required to restore single tooth gaps with bridges representing conventional tooth supported fixed partial dentures, implant therapy has become a reliable method chosen by more and more patients [4].

The history of dental implants can be traced back several centuries, whereas many kinds of implant materials were applied, e.g., porcelain, cobalt-chromium alloy, iridium and platinum until the osseointegration of titanium was discovered. After decades of research, the most commonly used implants are nowadays titanium-based [5]. Due to the immediately established oxide layer on the surface of titanium, which passivates the surface, titanium has good biological compatibility and osseointegration behavior [6]. However, 13% of patients with dental implants still have peri-implant inflammation. This chronic inflammation will gradually lead to the loss of bone around the implant, resulting in implant loss [7]. Some studies have shown that the corrosion products of titanium around implants are significantly increased in peri-implant inflammation [8]. The microscopic study of such peri-implantitis showed that they were associated with the presence of foreign bodies, such as titanium particles [9].

In addition to peri-implantitis, titanium also causes artifacts in X-ray imaging due to its metallic character [10].

Due to possible adverse reactions against metallic components such as the aforementioned titanium-associated peri-implantitis, there is a high demand for metal-free restorations [7-10].

Additionally, the mismatch between the elasticity of the implant material and the peri-implant bone could also be a major problem with current implants. The elastic modulus of titanium implants is approximately 110 GPa [11], while the elastic modulus of human trabecular and cortical bone are much lower (between 10.4 ± 3.5 GPa and 18.6 ± 3.5 GPa [12]). This mismatch could cause overloading of the peri-implant bone due to stresses at the bone-implant interface, thus damaging the peri-implant bone, resulting in implant loss [13].

As an alternative, the semi-crystalline high-performance thermoplastic polymer, PEEK (polyether ether ketone) representing a metal-free implant material with outstanding biocompatibility may replace metallic implant components [14]. PEEK has an elastic modulus of about 3-4 GPa, which can be adjusted with reinforcing carbon fibers so that the elastic modulus can be increased, e.g. to about 18 GPa, matching the elastic modulus of human trabecular and cortical bone [15]. Therefore, PEEK seems to have some biomechanical advantages when used as an implant material, e.g. in the field of oral and maxillofacial surgery, orthopedics and traumatology [16,17]. In the field of dentistry, PEEK is currently used as a framework material for dental prostheses [18]. According to the literature, there is a high interest in using PEEK as dental implant material [19].

However, the biological activity of the PEEK surface is low, and its capability to integrate into bone is low [20]. Therefore, the surface of PEEK must be modified to improve its biological activity. There are several surface treatment methods commonly used, such as coating, chemical and physical treatments[21]. Among these methods, plasma treatment has been increasingly used in recent years [22]. For example, plasma treatment of PEEK surfaces can significantly improve the bonding strength between a veneering composite and PEEK [23,24].Since, the chemical surface treatment approach generally requires the use of hazardous solutions such as concentrated sulfuric acid due to the high chemical stability of PEEK and the surface coating technology is relatively complicated, plasma treatment seems to represent a relatively convenient and safe alternative to improve the biocompatibility and overcome the bioinertness of PEEK [25] . Some articles have also shown surface disinfecting effects of plasma treatment [26]. Therefore, plasma treatment for implant surface modification

might be able to complete the disinfection process in the meantime, which would be conducive to large-scale production and probably cost saving.

Basically, different plasma treatments significantly increase the wettability and the ability of cell adhesion and proliferation of the PEEK surface [27-29]. After argon, nitrogen, and oxygen radio frequency plasma treatment, the self-bonding strength and hydrophilicity of PEEK are increased [27]. However, the increase in hydrophilicity caused by oxygen plasma treatment seems not to be stable, thus decreasing over time [28]. PEEK treated with water plasma immersion ion implantation seems to be more conducive to the adhesion and proliferation of osteoblasts [29]. Plasma immersion ion implantation treatment using methane and oxygen gas mixture also greatly improves the cell adhesion and diffusion ability of PEEK surface [30].

Besides those plasma treatments described in the literature, there is no article reporting on the effects of PEEK surfaces on osteoblasts after low-pressure plasma treatment (LPPT) as a simple and highly reproducible method.

Additionally, hydrogen, nitrogen, helium and argon plasma treatment can increase the surface hardness of PEEK to varying degrees [31,32]. In general, the hardness of PEEK is related to its crystallinity [33], which is directly influenced by the production process. For example, rapid cooling will result in a lower crystallinity [34]. Commonly, the degree of crystallinity of PEEK can be 0 - 47% [34]. The reason for the change in the surface hardness due to plasma treatment has not been analyzed on the atom level yet. Accordingly, there is no study reporting on possible changes in the surface hardness after LPPT.

Therefore, the purpose of this study was to analyze possible changes in the physical, chemical and biological properties of PEEK surfaces after the exposure to various low-pressure plasma processes.

2. Materials and methods

2.1 Preparation of PEEK specimens

PEEK was delivered in the shape of a round rod with a length of 1000 mm and a diameter of 14 mm (Evonik Industries AG, Essen, Germany). A saw with water-cooling (IsoMet 1000, Buehler, USA) was used to cut discs out of the semifinished good with a thickness of 3 mm. The PEEK discs were ground on one side with 320 grit sandpaper (Hermes Schleifmittel GmbH, Hamburg, Germany). Afterwards, the discs were pasted with the ground side on a plexiglass plate with double-sided tape in a group of 20 samples. The samples on the plexiglass plate could then be ground at one time using a grinding machine (Exakt 400 CS, Norderstedt, Germany) under water cooling washing away the grinding debris. The disc-shaped PEEK samples were ground with gradually reduced grit size of the sandpaper (320 grit, 800 grit, 1200 grit, 2500 grit and 4000 grit; Hermes Schleifmittel GmbH, Hamburg, Germany), for 10 min with each grit. After grinding, all specimens were rinsed with deionized water. Then the samples were stored in dry atmosphere at room temperature.

The PEEK samples (n=218) were divided into 4 groups according to treatment in a low-pressure plasma system (LPPT, Femto PCCE, Diener electronic GmbH & Co KG, Ebhausen, Germany) using different process gases:

1. Untreated PEEK (n=32, without LPPT)

2. H-PEEK (n=62, hydrogen LPPT)

3. O-PEEK (n=62, oxygen LPPT)

4. H/O-PEEK (n=62, hydrogen/oxygen LPPT, mixing ratio 2:1)

2.2 Low-pressure plasma treatment (LPPT)

After putting the PEEK samples into the process chamber of the low-pressure plasma device, the chamber was vented and the process gas was injected into the chamber. The gas in the chamber was discharged until a vacuum state was reached, followed by gas infill for more than 5 min until a stable pressure inside the process chamber of 0.4 mbar was created. Besides the low-pressure at 0.4 mbar, a temperature of 70°C and a process power of 200 W were used as further parameters for the LPPT. Each LPPT group was subdivided into four groups according to four different process durations (10s, 20s, 1 min, 5 min, 10 min and 30 min). The groups are summarized in Table 1.

Table 1: Overview of the different groups and the consecutive analyses.

	untreated PEEK	H-PEEK					O-PEEK						H/O-PEEK						SUM
Process time (s)	0	10	20	60	300	600	1800	10	20	60	300	600	1800	10	20	60	300	600	1800	
number of samples for water contact angle measurement after different LPPT durations (n)	9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	63
number of samples for water contact angle measurement over time (n)				21						21						21				63
number of samples for surface roughness measurement (n)	3						3						3						3	12
number of samples for FTIR and surface hardness measurement (n)	5						5						5						5	20
number of samples for water contact angle measurement after sterilization (n)	3						3						3						3	12
number of samples for cell fixation microscopy (n)	3						3						3						3	12
number of samples for cell fluorescence microscopy (n)	9						9						9						9	36
																				218

2.3 Water contact angle measurement

Directly after the LPPT, the water contact angle of the PEEK surfaces was measured using a digital microscope (Keyence VHX-5000, Neu-Isenburg, Germany). The objective of the microscope was set to a horizontal position so that the top of the PEEK specimens appeared as a horizontal line in the center of the microscopic image. A droplet of 10 µl deionized water was dropped on the sample surface. After 10 s, when the shape of the droplet was stable, a picture was taken. The integrated software was used to measure the water contact angle.

2.4 Surface roughness measurement

The roughness of the surfaces (Ra) was assessed using the Alicona infinite focus system (Alicona Imaging GmbH, Raaba, Austria). First, the PEEK surfaces were scanned three-dimensionally at 20-fold magnification. Along two 4 mm long vertical lines drawn on the 3d images, the surface roughness (Ra) was measured and the average roughness of the resulting values were calculated.

2.5 Fourier-transform infrared spectroscopy (FTIR)

To assess changes in the crystallinity of the surface of the samples, Fourier-transform infrared spectroscopy (FTIR Microscope Hyperion 3000, Bruker, Rheinstetten, Germany) was used after a plasma process duration of 30 min. The spectra range was between the wavenumbers 1800 cm^{-1} and 600 cm^{-1}. Since the ratio of the peaks at the wavenumbers 1305 cm^{-1} and 1280 cm^{-1} (1305 cm^{-1} / 1280 cm^{-1}) form the crystallinity index (CI [%]) [35], these peaks of the absorption bands were recorded. Based on the CI, the degree of crystallinity was obtained using the following formula (ASTM Standard F2778 - 09):

$$\text{Crystallinity}\,[\,\%\,] = \frac{CI - 0.728}{1.549} * 100$$

2.6 Surface micro-hardness measurement

The same batch of samples subjected to FTIR were also used to assess the surface micro-hardness. The micro-hardness of the sample surfaces was tested after LPPT for 30 min using a hardness testing device (Q10M, Qness GmbH, Golling, Austria) in accordance with ISO standard 6507 using a diamond with a tip angle of 136°. The used test forces were 0.005N, 0.01 N, 0.02 N, 0.2 N and 0.5 N. After the test, an integrated microscope was used to measure the length of the notch "L" to assess the indentation depth “D” indirectly using the following formula: $D = L / 2 \tan 68°$.

2.7 Water contact angle measurement after sterilization

After LPPT and before the cell culture experiments, the samples were sterilized in 60% isopropanol for 1 hour .

In order to reveal effects of the sterilization process on the hydrophilicity of the PEEK surfaces, three extra samples per group were used to measure the water contact angle after 30 min LPPT and subsequent sterilization with 60 % isopropanol for 1 hour.

2.8 Cell culture

Primary human osteoblasts (HOB; Provitro AG, Berlin, Germany) were stored in liquid nitrogen at a temperature of -196°C. A tube of frozen cells was taken out and quickly thawed in 37°C warm water. The thawed cell suspension was then transferred to the T25 cell culture flasks, and the osteoblast culture medium (Provitro AG, Berlin, Germany) was added, which was warmed up to the same temperature of 37°C in the incubator beforehand. The cell culture medium was first changed after one day of incubation. Afterwards the medium was changed after two to three days. When the cells were 80% confluent, they were transferred to three T25 cell culture flasks. After the passage to the third generation, cell culture tests were performed.

Therefore, the samples were placed in 24 well plates after 30 min LPPT and subsequent sterilization. Then, 2ml osteoblast culture medium containing 5 x 10^4 human osteoblasts was added to each well. The well plates were incubated at 37℃ in a humid atmosphere with 5 % CO_2.

2.9 Cell fixation microscopy

Six hours after seeding the cells, the samples were gently rinsed with PBS (phosphate-buffered saline) and placed in 75% ethanol for 10 min. Then, the samples were placed in ethanol with ascending concentration of 80%, 85%, 90%, 95% and 99% for 5 min each. They were then taken out and left to air dry. After fixation, images of the cells were obtained using a digital microscope (Keyence VHX-5000, Neu-Isenburg, Germany).

2.10 Fluorescence microscopy

The osteoblast culture medium was changed after 3 and 6 days. On days 1, 3, and 7 after cell seeding, the area of the PEEK surface covered by osteoblasts was detected using a fluorescence microscope.

Fluorescein diacetate (FDA; Sigma-Aldrich, Taufkirchen, Germany) was dissolved in acetone to obtain a 5 mg/ml stock solution. Then, a working solution was prepared consisting of, 5 µl stock solution per ml PBS. After washing the specimens with sterile PBS, 1ml of the working solution was added and the specimens were incubated at 37°C for 30 min. Afterwards, the specimens were washed again with PBS and directly evaluated with a fluorescence microscope (Vanox-T, Olympus, Hamburg, Germany).

For this purpose, excitation/emission wavelengths of 490/526 nm were used. Of each specimen, 3 pictures of the surface were captured, each corresponding to an area of 3.72 mm^2 (2.24 x 1.66 mm), which were located around the center of the sample surface in a triangular manner. The pictures were then analyzed with Image J (National Institutes of Health, USA) to calculate the area covered by the cells.

2.11 Statistical analysis

For statistical analysis, the normality, the homogeneity test (Levene's test) and the one-way ANOVA were performed to detect significant differences in results, commercial software was used (OriginLab Corporation, Northampton, USA). The significance level in the results was set at $p<0.05$.

3. Results

3.1 Water contact angle measurement after different LPPT durations

The results of the water contact angle measurements are shown in Figure 1. The contact angle of untreated PEEK was 80.33 ± 1.12°, representing the result at 0 s treatment time in Figure 1. After 1 min (60 s) of LPPT, the surface contact angle showed the biggest change. When the LPPT time was longer than 10 min (600 s), the contact angle of O-PEEK and H/O-PEEK almost approached 0°, whereas H-PEEK showed a water contact angle of 41.67 ± 1.15°, which changed non-significantly (41.00 ± 2.65°) after a treatment time of 30 min (1800 s). After a LPPT time less than 10 min (600 s), the groups could be ranked according to their water contact angles as follows: H-PEEK > O-PEEK > H/O-PEEK. After a LPPT time of 10 s, 20 s, and 60 s, differences between the results of the 3 LPPT groups were significant ($p<0.05$). After a process time longer than 5 min (300 s), the results of the O-PEEK and H/O-PEEK groups did not differ significantly ($p>0.05$), but they differed significantly from the results of the H-PEEK group ($p<0.05$).

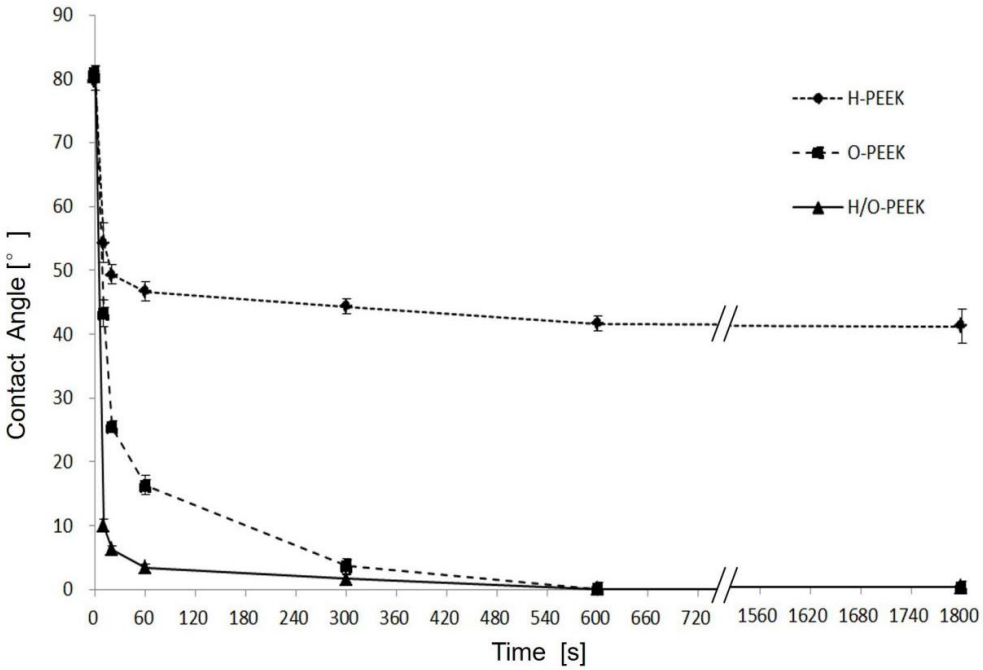

Figure 1: Contact angles of the different groups after different LPPT durations [23]

3.2 Water contact angle over time

The developments of contact angle in air over time after LPPT are shown in Figure 2. Starting from the contact angles after 1 min (60 s) of LPPT, the contact angles of the three groups

gradually increased and reached a stable plateau after about one week. On day 14, the contact angle of H-PEEK was 64.00° ± 1.00°, the contact angle of O-PEEK was 37.67° ± 1.53°, the contact angle of H/O-PEEK was 27.67° ± 0.58°. These results differed significantly from each other and the untreated PEEK group ($p<0.05$).

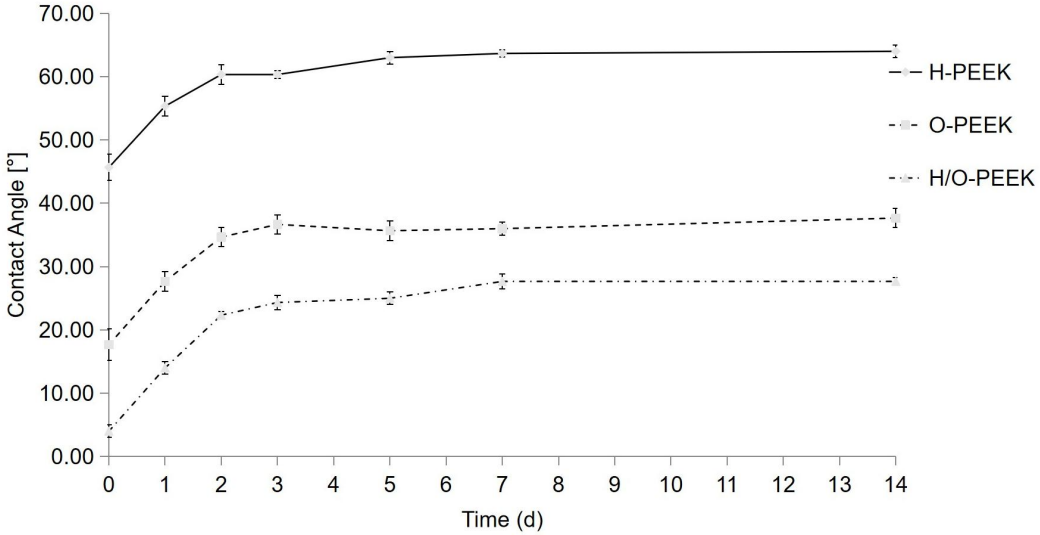

Figure 2: Development of the water contact angles over time after 60s LPPT.

3.3 Surface roughness measurement

The results of the surface roughness measurements are shown in Figure 3. The roughness (Ra) of untreated PEEK was 0.41 ± 0.07 µm, the roughness of H-PEEK was 0.42 ± 0.07 µm, the roughness of O-PEEK was 0.40 ± 0.07 µm and the roughness of H/O-PEEK was 0.43 ± 0.06 µm. There was no significant difference between the 4 groups ($p>0.05$).

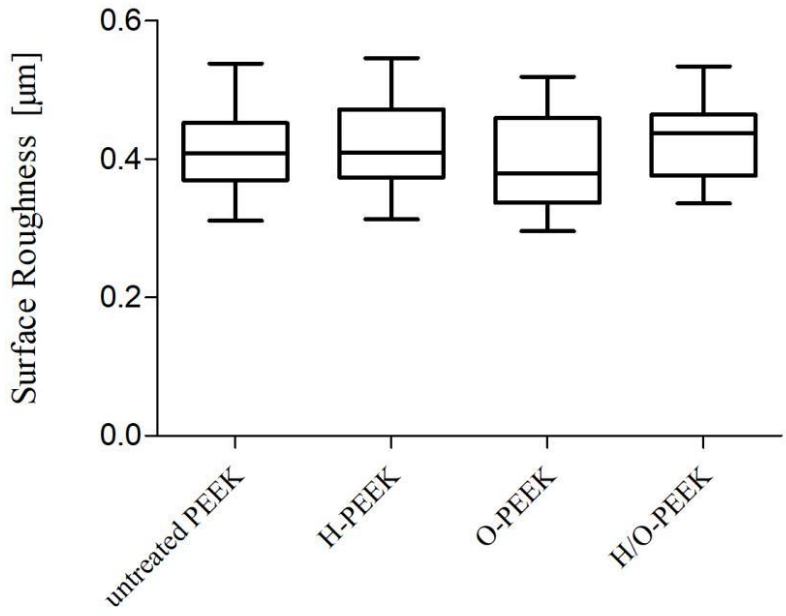

Figure 3: Surface roughness of the different groups after LPPT for 30 min [23]

3.4 FTIR

For all groups, the "b peaks" at a wavelength of 1280 cm^{-1} showed the same height, whereas the "a peaks" at a wavelength of 1350 cm^{-1} differed significantly between the groups ($p<0.05$; Figure 4A). Therefore, the CI of the untreated PEEK group was 87.43 ± 0.95 %, of the H-PEEK group 91.39 ± 0.96 %, of the O-PEEK group 100.41 ± 2.11 % and of the H/O-PEEK 110.44 ± 2.51 % (Figure 4B). Based on the CI, the untreated PEEK group showed a crystallinity of 9.45 ± 0.61 %, the H-PEEK group 12.00 ± 0.62 %, the O-PEEK group 17.83 ± 1.36 %, and the H/O-PEEK group 24.30 ± 1.62 %. These results were significantly different ($p<0.05$).

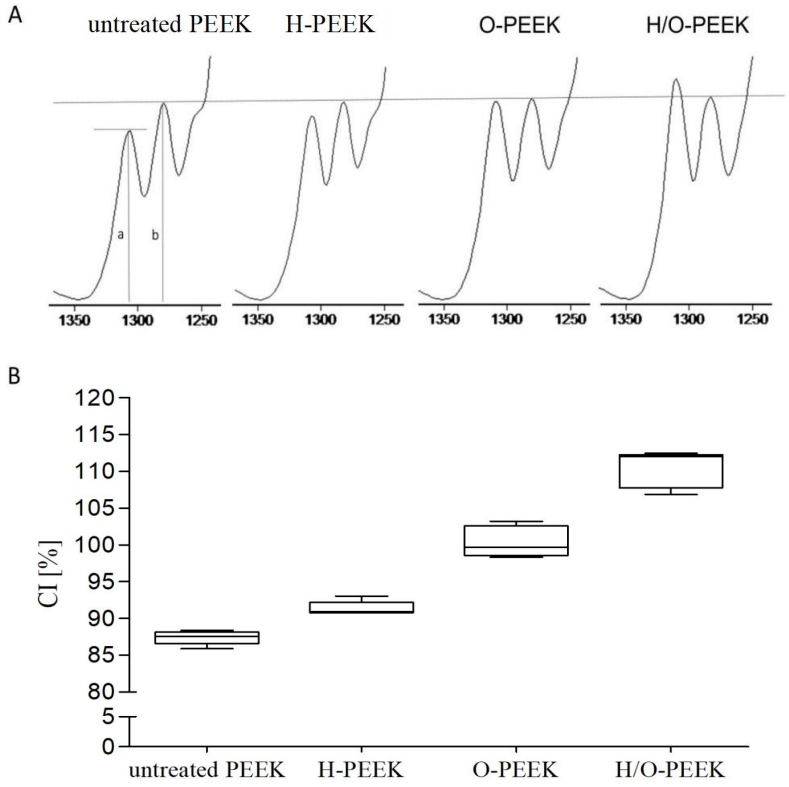

Figure 4: (A) FTIR measurements of the different groups between the wavelengths 1250 and 1350 cm^{-1}, showing the "a" and "b peak" at wavelength 1350 cm^{-1} and 1280 cm^{-1} respectively; (B) Graphical illustration of the CI of each group, which were significantly different ($p<0.05$) [23].

3.5 Surface micro-hardness measurement

The results of surface micro-hardnesses are shown in Table 2 and Figure 5. When the test force increased, the difference in micro-hardness between the groups decreases. When the test force was 0.02 N and less, there was a significant difference between the surface

micro-hardness of each group ($p<0.05$), and the surface micro-hardness could be positively correlated with the surface crystallinity in the same order like the crystallinity (PEEK < H-PEEK < O-PEEK < H/O-PEEK). At a test load of 0.2 N, the results did not differ significantly between the untreated PEEK group and the H-PEEK group, and between the O-PEEK group and the H/O-PEEK group ($p>0.05$). The relationship between the indentation depth and the micro-hardness was the same.

There was no significant difference between the groups when a higher force of 0.5 N was used ($p>0.05$).

In terms of indentation depths, untreated PEEK showed the highest result, followed by H-PEEK, O-PEEK and H/O-PEEK, with results differing significantly between groups when a test load of 0.02 N and less was used ($p<0.05$). When a test force of 0.5 N was used, all samples showed an indentation depth of 14 µm.

Table 2: Results of the micro-hardness measurements [23].

	Test Forces	untreated PEEK	H-PEEK	O-PEEK	H/O-PEEK
Micro-hardness [N/mm^2]	0.005 N	182.67 ± 2.4 [a]	229.90 ± 6.8 [a]	268.12 ± 6.8 [a]	289.49 ± 7.5 [a]
	0.01 N	183.26 ± 1.5 [a]	213.83 ± 2.8 [a]	231.67 ± 2.6 [a]	258.52 ± 2.8 [a]
	0.02 N	183.26 ± 1.5 [a]	200.90 ± 2.2 [a]	212.27 ± 2.6 [a]	228.14 ± 1.9 [a]
	0.2 N	182.28 ± 2.7 [b,c]	183.26 ± 2.6 [d,e]	187.96 ± 2.3 [b,d]	188.75 ± 3.2 [c,e]
	0.5 N	182.67 ± 2.4	182.48 ± 2.1	182.67 ± 2.6	182.67 ± 2.3
Indentation depth [µm]	0.005 N	1.41 ± 0.02 [a]	1.20 ± 0.04 [a]	1.16 ± 0.03 [a]	1.02 ± 0.03 [a]
	0.01 N	2.01 ± 0.02 [a]	1.83 ± 0.02 [a]	1.75 ± 0.02 [a]	1.62 ± 0.02 [a]
	0.02 N	2.82 ± 0.02 [a]	2.70 ± 0.03 [a]	2.61 ± 0.03 [a]	2.51 ± 0.02 [a]
	0.2 N	10.13 ± 0.15 [b,c]	10.07 ± 0.14 [d,e]	9.81 ± 0.06 [b,d]	9.78 ± 0.16 [c,e]
	0.5 N	14.05 ± 0.18	14.06 ± 0.16	14.05 ± 0.20	14.05 ± 0.17

[a] significantly different results of all groups ($p<0.05$).

[b] significantly different results of untreated PEEK and O-PEEK ($p<0.05$).

[c] significantly different results of untreated PEEK and H/O-PEEK ($p<0.05$).

[d] significantly different results of H-PEEK and O-PEEK ($p<0.05$).

[e] significantly different results of H-PEEK and H/O-PEEK ($p<0.05$).

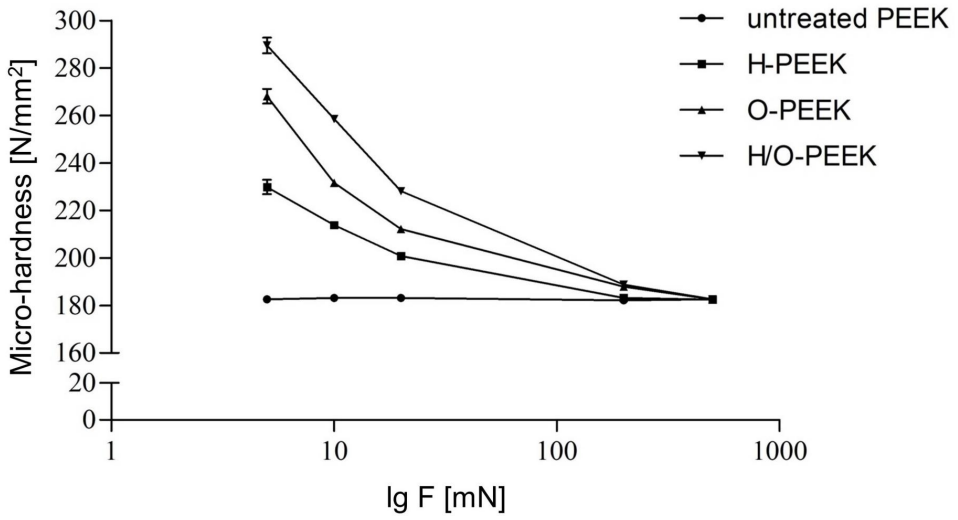

Figure 5: The micro-hardnesses of different LPPT groups under different test forces [23].

3.6 Water contact angle measurement after sterilization

After sterilizing the specimens for 1 hour with 60 % isopropanol, the water contact angles of the PEEK surfaces increased significantly, but the ranking of the groups did not change (Figure 6). Before and after sterilization, there was no significant difference between the results of the O-PEEK group and the H/O-PEEK group ($p>0.05$), but they were significantly different from the results of the H-PEEK group ($p<0.05$).

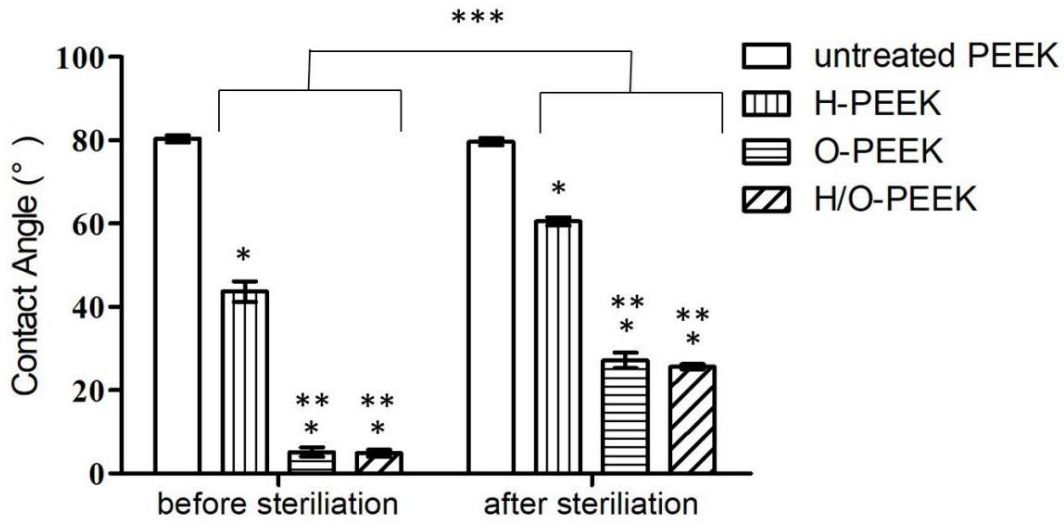

Figure 6: Results of the water contact angle measurements before and after sterilizing [23].

*significant difference compared to untreated PEEK ($p<0.05$);

** significant difference compared to H-PEEK ($p<0.05$);

*** significant difference between the results before and after the sterilization process ($p<0.05$).

3.7 Cell fixation microscopy

Figure 8 shows the different cell morphologies due to the different groups after 6 hours of human osteoblasts seeding. On untreated PEEK, the cells showed a spherical morphology with a small mass. On H-PEEK, a small number of pseudopodia appeared which expanded slightly. On O-PEEK, the cells developed more pseudopodia and the cells were larger. In the H/O-PEEK group, human osteoblasts showed the largest number of pseudopodia in combination with the largest cell size and thus the most advanced cell adhesion ability after 6 hours.

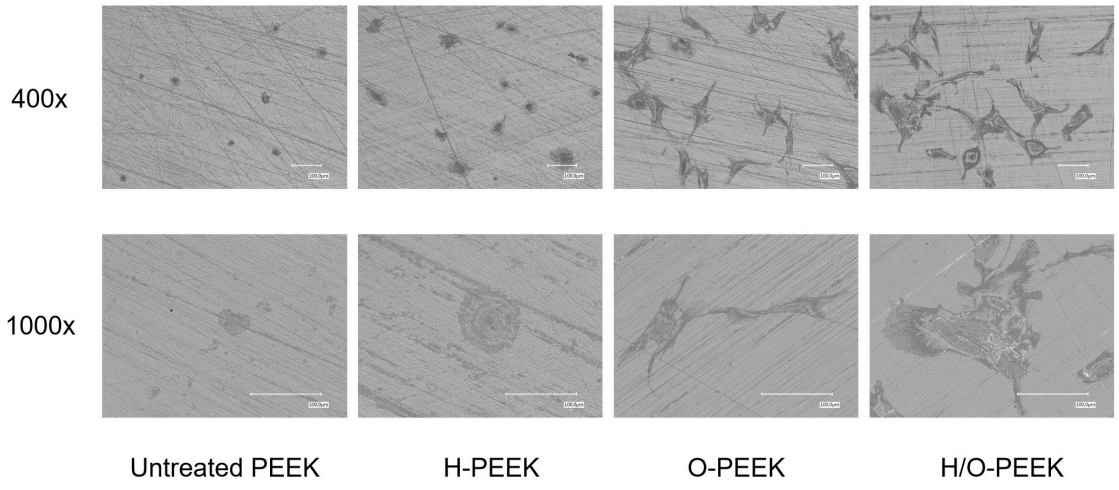

Figure 7: Digital microscopy images of human osteoblasts on the sample surfaces after 6 hours.

3.8 Fluorescence microscopy

Figure 8 shows pictures deriving from the fluorescence microscopy of human osteoblasts on the surfaces of PEEK after cultivation for 1, 3 and 7 days. Figure 7 shows the calculated proportion of the surface of PEEK covered by the human osteoblasts. In general, plasma treated specimens showed a significantly higher number of cells than the untreated PEEK group ($p<0.05$). After 1 week (7 days), the O-PEEK and H/O-PEEK samples were the most covered with cells, about 40 % of their surfaces, followed by the H-PEEK group with around 20% (Figure 9). The results of the O-PEEK group and the H/O-PEEK group did not differ significantly ($p>0.05$), but they were significantly different from the results of the H-PEEK group ($p<0.05$).

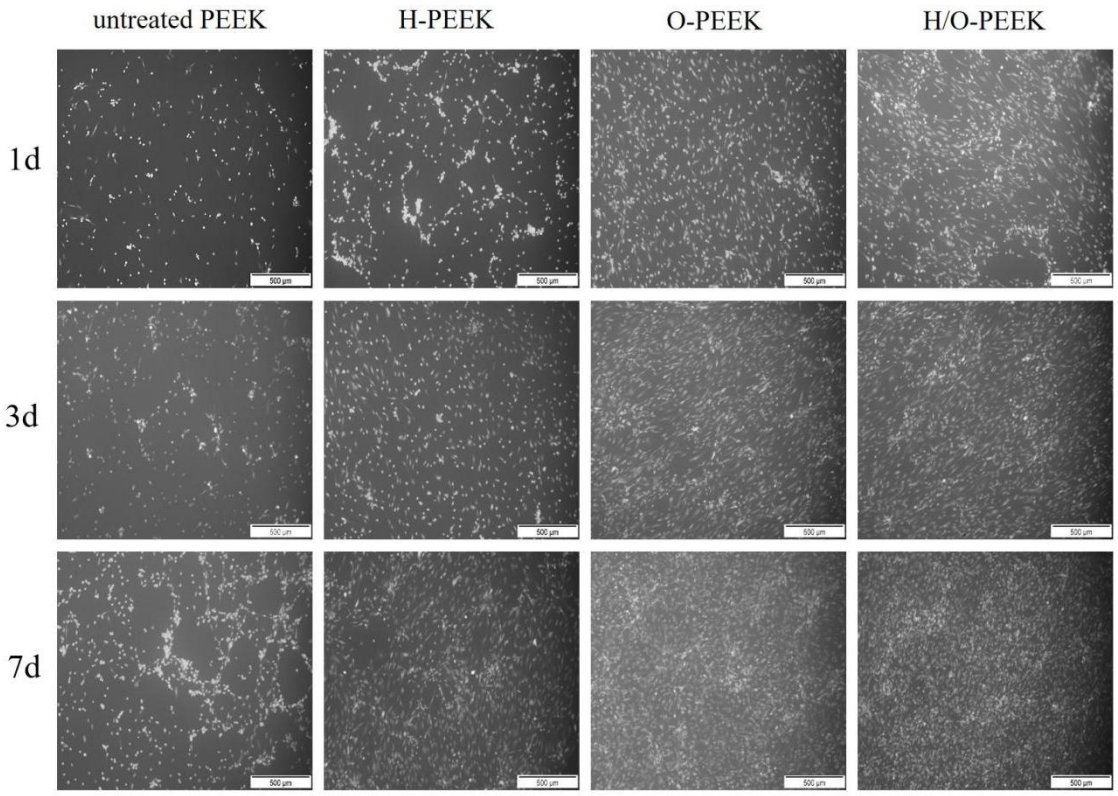

Figure 8: Fluorescence microscopy images of human osteoblasts on the sample surfaces [23].

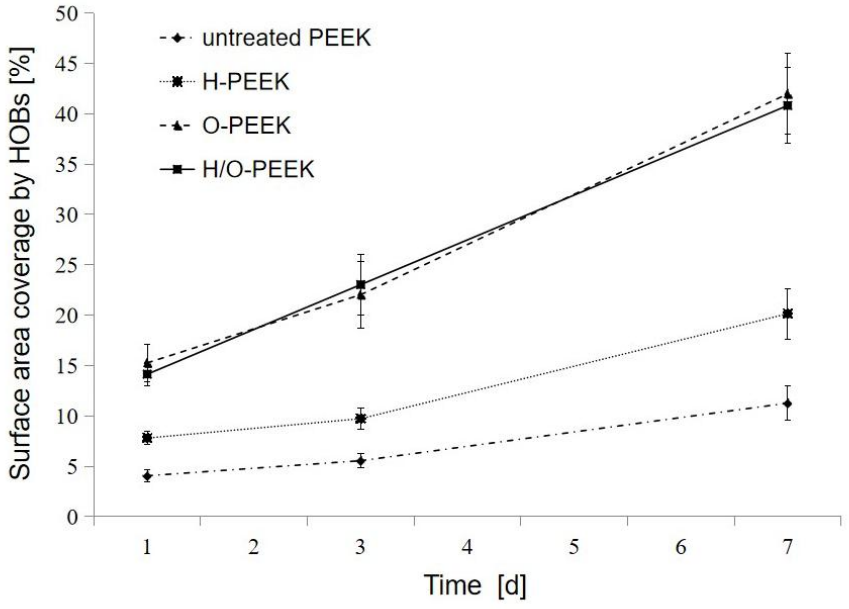

Figure 9: Surface area coverage by human osteoblasts [23].

4. Discussion

The results of the present study showed that different LPPTs can significantly improve the hydrophilicity, crystallinity, micro-hardness and proliferation activity of human osteoblasts, similar to the results described in the literature [27-33].

In general, a high surface roughness seems to be important for adhesion of osteoblasts and osteogenesis [36], whereas in the present investigation, PEEK surfaces did not show different roughnesses due LPPT. Insofar, the increase in cell proliferation after LPPT may be due to changes of the chemical composition of the specimen surfaces, especially of the O-PEEK and H/O-PEEK groups. Although the contact angle was increased after sterilizing and long-term storage, the O-PEEK group and H/O-PEEK group still showed significantly enhanced biological activities and hydrophilicity compared to untreated PEEK. Therefore, the use of oxygen or a hydrogen/oxygen mixture as a process gas for LPPT could be a feasible method to improve the biological activity of PEEK implants. Theoretically, the process of propanol sterilization could have been omitted, because the LPPT itself has a sterilization effect [37], which would result in an even higher cell adhesion effect. As after 10 min of LPPT, the contact angles of the samples treated with oxygen and hydrogen/oxygen LPPT were close to 0°, cell adhesion and proliferation might have been similar after a LPPT time of 10 min, since a longer duration did not have an additional impact on the water contact angles.

As titanium is still the most commonly used material for endosseous implants, the non-inclusion of this material must be considered a limitation. In addition, subsequent studies should evaluate other cell reactions, such as cell viability and cell proliferation rate due to LPPT.

The present study showed that the effects of LPPT were strongly depending on the gas used, whereas the gases could be ranked according to their influence strengths as follows: hydrogen < oxygen < hydrogen/oxygen. Besides the etching and cleaning effects, LPPT might also change the chemical composition of PEEK surfaces, thereby changing its surface properties.

During LPPT, high-energy ions and free radicals bombarding the sample surface will cause localized heating at the nanometer level and activate chemical reactions [38,39]. In this study, plasma radicals of small H-atoms and larger O-atoms were used. They can cause different changes in the surface of PEEK in terms of ablation and chemical modification. According to the literature, the use of humid air plasma to treat polypropylene can increase the densities of acid, carbonyl, peroxy radicals and alcohol on its surface [38]. After the treatment with

oxygen, carbon acid bonds could be detected on the surface of PEEK [28]. Besides these, also –OH groups could be found after oxygen LPPT treatment [39].

Thus, using hydrogen LPPT might have not only broken the C–O–C bond, but also the C=O bond within the surface of polyether ether ketone, thereby forming C–OH. During this process, some benzene rings may have been split and evaporated (Figure 10). Therefore, the samples could have presented a higher amount of C–OH groups, what might have caused the higher hydrophilicity. In addition, the relatively high water contact angle could have been due to missing polar groups like during oxygen LPPT.

During oxygen LPPT, C–O–C bonds may be broken and removed and O-atoms/radicals may be introduced to create C–O–O–C. Unstable radicals like O=C–O • and C–O • could have been created, deriving from the breakup of the bonding between benzene rings and C=O groups (Figure 10). After the oxygen LPPT, these unstable radicals could have reacted with water in the air to form O=C–OH and C–OH, which could have made the surface of PEEK more hydrophilic than hydrogen LPPT.

In addition, preliminary tests showed that the water contact angle after hydrogen LPPT varied between 10° and 45° depending on the weather. Because it had a small angle on rainy days and a large angle on sunny days. The possible reason was that the machine cannot vacuum all the air, and the remaining air has an impact on the result of the plasma treatment. In this respect, the pump-down time for vacuum formation was extended and the gas filling phase was increased to 5 min to eliminate the environmental influences, whereupon uniform water contact angles were achieved.

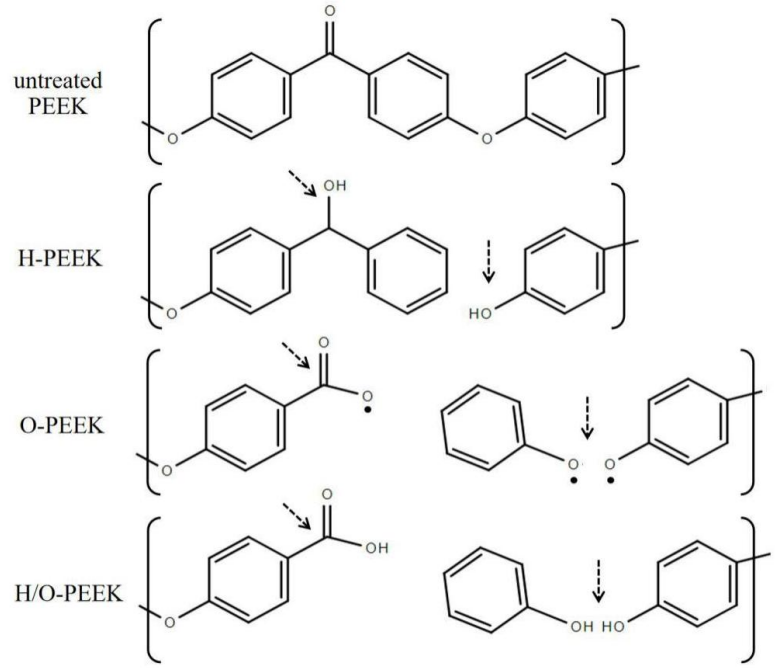

Figure 10: Theoretic chemical reactions of PEEK induced by LPPT [23].

Regarding the hydrogen/oxygen LPPT, this reaction could have been occurring while the LPPT was still taking place, since H and O radicals were available at the same time. Although this gas mixture may explode easily under normal conditions, the application for this study was safe, as it was used at a very low pressure. The use of this gas mixture is officially approved by the producer and the plasma chamber is equipped with a safety valve.

When comparing the development of the water contact angles over treatment time of both, oxygen and hydrogen/oxygen LPPT, it could be assumed that the hydrogen/oxygen LPPT was more effective in terms of forming more hydrophilic groups in less time.

Moreover, the newly formed O=C–OH and C–OH bonds could have reacted forming new chemical groups during LPPT, which could have been caused the higher degree of crystallinity and the accordingly higher surface micro-hardness of the samples. In case PEEK is used as implant material, the increased surface micro-hardness might protect the the surface layer during insertion.

When analyzing the dependence of crystallinity on micro-hardness, a considerable exponential correlation became evident (Figure 11). Accordingly, the crystallinity (y) [%] can approximately be calculated by the following formula when the micro-hardness (H) [N/mm^2] is known:

$y = 1.8449e^{0.0086H}$

This equation was associated with a high coefficient of determination $R^2 = 0.9265$. Therefore, using this formula together with micro-hardness test could represent a simple alternative to the rather complicated FTIR method to determine the crystallinity of PEEK surfaces. For this, the ideal load force would be 0.005 N.

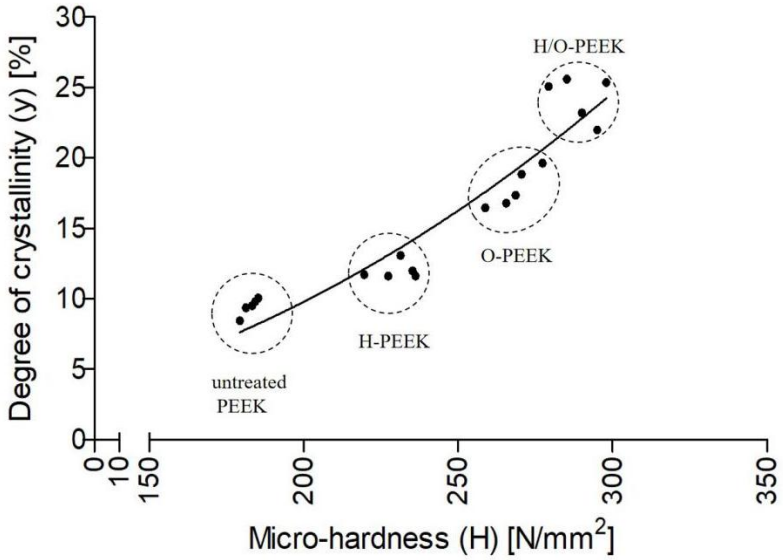

Figure 11: The dependence of micro-hardness on crystallinity after LPPT [23].

Theoretically, the cooling rate during manufacturing [34] and the temperature during compression molding influences the crystallinity degree of PEEK [36]. A mold temperature of 340 °C, led to a crystallinity of 23 %, a higher mold temperature of 390 °C caused a lower degree of crystallinity of 14 % [35]. The crystallinity test result of the untreated PEEK group was 9.18 %. This quite low result could be caused by the preparation of the PEEK specimens, where cutting and grinding could have caused local heat despite the specimens were cooled by water.

Regarding the crystallinity, more different LPPT times should be evaluate in future experiments to determine the process duration causing the maximum crystallinity in the surface of PEEK.

Additionally, these modified surfaces should undergo tribological experiments to assess their wearing properties, what would be interesting considering the insertion process of a dental implant [40] and PEEK as a potential dental implant material.

In addition, the positive results of the in-vitro cell culture tests with human osteoblasts need to be verified in vivo.

5. Conclusion

The present study showed that LPPT enhanced the hydrophilicity, crystallinity, micro-hardness, cell adhesion and proliferation of the surface of PEEK. Among the process gases evaluated, a mixture of hydrogen and oxygen in a 2:1 ratio was most effective.

6. References

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Statutory Declaration

“I, Qian, Fu, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “**The impact of different low-pressure plasma types on the physical, chemical and biological surfaceproperties of PEEK / Der Einfluss unterschiedlicher Niederdruckplasmabehandlungen auf die physikalischen, chemischen und biologischen Oberflächeneigenschaften von PEEK**”, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

[In the case of having conducted your doctoral research project completely or in part within a working group:] Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of your own contribution to the publications

Qian Fu contributed the following to the below listed publications:

Publication: Fu Q, Gabriel M, Schmidt F, Müller WD, Schwitalla AD. The impact of different low-pressure plasma types on the physical, chemical and biological surface properties of PEEK. Dent Mater. 2021 Jan;37(1):e15-e22.

Contribution: I am responsible for completing the collection and analysis of all figures and tables in the article. All the raw data related to the publication were obtained by my own. I wrote the article, and other authors modified the content of the article.

_____
Signature, date and stamp of first supervising university professor / lecturer

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Signature of doctoral candidate

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9	Clinical Implant Dentistry and Related Research	4,496	3.396	0.008270
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12	International Journal of Oral Science	1,204	3.047	0.002020
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14	Molecular Oral Microbiology	993	2.905	0.001720
15	Clinical Oral Investigations	6,849	2.812	0.011470
16	JOURNAL OF THE AMERICAN DENTAL ASSOCIATION	6,967	2.803	0.004990
17	Journal of Prosthodontic Research	1,283	2.662	0.002150
18	European Journal of Oral Implantology	1,227	2.619	0.002230
19	ORAL DISEASES	4,463	2.613	0.005080

Print copy of the selected publication

Qian Fu, Matthias Gabriel, Franziska Schmidt, Wolf-Dieter Müller, Andreas Schwitalla*. The impact of different low-pressure plasma types on the physical, chemical and biological surface properties of PEEK. Dental Materials. Jan 2021, Vol 37(1), e15-e22.

https://doi.org/10.1016/j.dental.2020.09.020

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Complete list of publications

1. **Qian Fu**, Matthias Gabriel, Franziska Schmidt, Wolf-Dieter Müller, Andreas Schwitalla*. The impact of different low-pressure plasma types on the physical, chemical and biological surface properties of PEEK. Dental Materials. Jan 2021, Vol 37(1), e15-e22.
→Journal Impact Factor 2019: **4.495**

2. Fuwei Liu#, **Qian Fu**# (Co-First author), Yunpeng Li, Kai Zhang, Mingyue Tang, Wei Jiang, Bin Bo*, Yajun Cui, Liang Kong*. USP21 Modulates Goosecoid Function through Deubiquitination. Bioscience Reports.2019,39(7): BSR20182148.
→Journal Impact Factor 2019: **2.94**

3. **Qian Fu**, Bellare Anuj, Yajun Cui, Bingkun Cheng, Shanshan Xu, Liang Kong*. The effect of hierarchical micro/nanotextured titanium implants on osseointegration immediately after tooth extraction in beagle dogs. Clin Implant Dent Relat Res. 2017 Jun;19(3):486-495.
→Journal Impact Factor 2019: **3.40**

4. Yongfeng Li#, **Qian Fu**# (Co-First author), Yaping Qi, MingMing Shen, Qiang Niu, Kaijin Hu*, Liang Kong*. Effect of hierarchical hybrid micro/nanorough strontium-loaded surface on osseointegration in osteoporosis. RSC Advances. 2015, 5, 52296 – 52306.
→Journal Impact Factor 2019: **3.12**

5. Yongfeng Li#, Yaping Qi#, Qi Gao#, Qiang Niu, Mingming Shen, **Qian Fu**, Kaijin Hu, Liang Kong*. (2015). Effects of a micro/nano rough strontium-loaded surface on osseointegration. International journal of nanomedicine, 2015; 10: 4549 – 4563.
→Journal Impact Factor 2019: **5.12**

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