Mechanical and biological characteristics of diamond-like carbon coated poly aryl-ether-ether-ketone

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A B S T R A C T

Poly aryl-ether–ether-ketone (PEEK) is an alternative to metal alloys in orthopedic applications. Although the polymer provides many significant advantages such as excellent mechanical properties and non-toxicity, it suffers from insufficient elasticity and biocompatibility. Since the elastic modulus of diamond-like carbon (DLC) is closer to that of cortical bone than PEEK, the DLC/PEEK combination is expected to enhance the stability and surface properties of PEEK in tissue replacements. In this work, PEEK is coated with diamond-like carbon (DLC) by plasma immersion ion implantation and deposition (PIII&D) to enhance the surface properties. X-ray photoelectron spectrometry (XPS), Raman spectroscopy, and Fourier transform infrared (FTIR) spectroscopy demonstrate successful deposition of the DLC film on PEEK without an obvious interface due to energetic ion bombardment. Atomic force microscopy (AFM) and contact angle measurements indicate changes in the surface roughness and hydrophilicity, and nanoindentation measurements reveal improved surface hardness on the DLC/PEEK. Cell viability assay, scanning electron microscopy (SEM), and real-time PCR analysis show that osteoblast attachment, proliferation, and differentiation are better on DLC/PEEK than PEEK. DLC/PEEK produced by PIII&D combines the advantages of DLC and PEEK and is more suitable for bone or cartilage replacements.

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1. Introduction

With increasing human longevity, more health care issues are looming and faster development of novel biomaterials, particularly tissue and organ substitutes, is imperative. Besides good mechanical properties and interfacial biocompatibility, biomaterials used in bone and joint replacements must offer good tissue tolerance without producing cytotoxic, carcinogenic, or mutagenic effects and provoke desirable biological responses in vivo [1]. The most popular orthopedic materials are metals such as titanium and its alloys which possess favorable attributes such as bio-inertness, high resistance to fatigue, and non-toxicity [2–5]. However, metals and alloys have some drawbacks. For instance, the elastic moduli of metal alloys are approximately 6–20 times greater than that of bones [6–8] causing bone resorption [9,10]. Moreover, the radiopacity of metal alloys and release of harmful metal ions in vivo are of concern. Therefore, it is necessary to develop better biomaterials for applications such as bone or cartilage grafting.

Polymers are receiving more attention as substitutes for metal alloys in orthopedic applications [9–12]. In particular, poly aryl-ether–ether-ketone (PEEK) with the basic structure of \((-C_6H_4-O-C_6H_4-O-C_6H_4-O-\)) is one of the candidates. PEEK is a linear polyaromatic and semicrystalline thermoplastic polymer boasting a suitable combination of high strength, stiffness, fatigue, and wear resistance [13]. In addition, it is easy to process, non-toxic [14] while possessing natural radiolucency as well as excellent thermal and chemical stability [15]. The elastic modulus of PEEK is 5 GPa which is closer to that of cortical bone (17 GPa) than titanium alloys (105–120 GPa). Consequently, the effects of stress shielding after implantation can be reduced if PEEK is used to substitute for metal alloys. Unfortunately, being bio-inert and hydrophobic has hitherto limited its applications.

The surface properties of biomaterials can be modified [16–19], and plasma immersion ion implantation and deposition (PIII&D) is particularly useful as a surface modification technique due to its simple operation and non-light-of-sight characteristics which bode well for biomedical implants with a complex shape [20,21]. Diamond-like carbon (DLC) films which have been demonstrated to be biocompatible both in vitro [22,23] and in vivo [24] possess many other desirable properties including excellent hardness, high stability, favorable tribological properties, and so on [25]. In this work, PIII&D is used for the first time to coat PEEK with DLC. The surface properties of DLC/PEEK are investigated and compared and the osteoblast behavior is also studied systematically.
2. Materials and methods

2.1. Sample preparation

Implantable grade PEEK (Ketron LSG, Quadrant EPP, USA) was machined into round samples with a diameter of 5 mm and thickness of 3 mm, polished, and ultrasonic cleaned before DLC deposition by PIII&D. Prior to film deposition, the substrates underwent Ar⁺ sputter cleaning for 3 min to remove surface contaminants and oxide. The base pressure in the vacuum chamber was 1 × 10⁻⁵ Torr. A mixture of acetylene (C₂H₂) and argon was subsequently bled into the chamber at an Ar to C₂H₂ flow rate ratio of 5:20 (sccm), and the plasma was triggered using radio frequency (RF). Film deposition was carried out at a constant RF power of 200 W and pressure of 8 × 10⁻⁴ Torr. The pulse duration was 200 μs and the repetition rate was 40 Hz. A negative bias voltage ~5 kV was applied to the substrates during deposition to improve film adhesion via ion mixing.

2.2. Surface characterization

X-ray photoelectron spectroscopy (XPS) was conducted on a Physical Electronics PHI-5802 to determine the composition and chemical structure of the samples. Elemental depth profiles were obtained by XPS using argon ion bombardment at an approximate sputtering rate of 10 nm/min. The core peaks of C₁s and O₁s were recorded and analyzed every minute.

Raman spectra excited by a 633 nm Ar⁺ laser were acquired to investigate the structural characteristics of the DLC films. The spectra were processed by Gaussian curve fitting and linear background subtraction. The molecular structure in the DLC was determined by a Perkin Elmer Spectrum One Fourier Transform Infrared (FTIR, ATR mode) spectrometry.

An atomic force microscope (AFM) made by Park Scientific Instruments/Auto Probe CP was used to evaluate the surface morphology of the DLC/PEEK and PEEK control (without DLC). The AFM images were obtained using the contact mode and the root-mean-square roughness (RMS) was determined by averaging results obtained from five different areas.

To determine the surface hydrophilicity, static contact angle measurements using distilled water and ethylene glycol as the media were performed by the sessile drop method on a Ramé-Hart (USA) instrument at ambient humidity and temperature. The drop size was 10 μl and each data point is the average of five measurements conducted on different parts of each specimen. Statistical analyses were performed by the student's t-test.

The hardness and elastic modulus values were determined by nanoindentation measurements using a three-sided pyramidal diamond (Berkovich) indenter with

![Fig. 1. XPS depth profiles of DLC/PEEK sample.](image1)

**Fig. 1.** XPS depth profiles of DLC/PEEK sample.

![Fig. 2. C₁s and O₁s spectra acquired from the DLC film and PEEK substrate: (a) C₁s at sputtering time of 3 min; (b) C₁s at sputtering time of 120 min; (c) Shift of O₁s from 3 min to 120 min.](image2)

**Fig. 2.** C₁s and O₁s spectra acquired from the DLC film and PEEK substrate: (a) C₁s at sputtering time of 3 min; (b) C₁s at sputtering time of 120 min; (c) Shift of O₁s from 3 min to 120 min.
options for continuous stiffness measurement. A series of indentations were performed in the depth range from 50 nm to 900 nm. Five indents were averaged to determine the mean $H$ value for each load to improve the statistics.

2.3. Cell culture

Cells of the human fetal-osteoblast cell line (hFOB 1.19, ATCC® Number: CRL-11372TM) were cultured in a 1:1 mixture of Ham’s F12 medium and Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The rat calvaria osteoblasts were obtained by sequential trypsin-collagenase digestion on calvaria of neonatal (<1 day old) Sprague-Dawley rats and cultured in the Dulbecco’s modified Eagle’s medium supplemented with 10% newborn bovine serum. Both kinds of cells were incubated in a humidified atmosphere of 5% CO$_2$. For the tests on cells viability and morphology, the hFOB 1.19 cells were laid on the specimens on 24-well tissue culture plates at a density of $1.5 \times 10^4$ cells/well. Before cell culturing, all the substrates were sterilized with 75% alcohol overnight and then rinsed with sterile phosphate-buffered saline (PBS) thrice. The culture media were refreshed every 3 days.

2.3.1. Cell viability

A cell counting kit (CCK-8 Beyotime, China) was employed to quantitatively identify the viable osteoblasts on the substrates after different time points of 6 h, 3 days, and 6 days. It is based on the cleavage of the tetrazolium salt WST-8 (4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1.3-benzene disulfonate) by mitochondrial dehydrogenases in metabolically active cells [26]. The harvested specimens were rinsed twice with sterile PBS and transferred to the fresh 24-well tissue culture plates. Afterwards, the CCK-8 solution was diluted with the growth medium and incubated with the samples in a separate volume of 0.7 ml for 4 h. Finally, the diluted solution incubated was aspirated and then measured spectrophotometrically at 450 nm. The data were presented as means and standard deviations of four parallel replicates for statistical accountability. The statistical analyses were performed by one-way ANOVA.

2.3.2. Cell morphology

The morphology of the osteoblasts after 6 h of incubation was inspected by scanning electron microscopy (SEM, JOEL JS-820). The samples were fixed with 2% polyoxymethylene and then dehydrated in a graded ethanol series (10, 30, 50, 70, 80, 95, 95% v/v) for 15 min, respectively. Final dehydration was conducted in absolute ethanol twice and then the substrates were dried in hexamethyldisilazane (HMDS) [27]. The dry specimens were sputter coated with gold to alleviate possible sample charging before SEM observation.

2.3.3. Quantitative real-time PCR

The osteogenic-associated genes of rat calvaria osteoblasts cultured were quantitatively analyzed by real-time PCR. After incubation on the various substrates for 3, 6 and 12 days, the total RNA of rat calvaria osteoblasts was isolated by using a TRIZOL reagent (Invitrogen) according to the manufacturer’s protocol. After isopropanol precipitation, the RNA pellet acquired was washed with 75% ethanol, treated with the diethyl pyrocarbonate (DEPC, RNAse inhibitor), and then solubilized in sterile DEPC water for concentration determination. A PrimeScript 1st Strand cDNA Synthesis kit (TaKaRa) was used to reverse transcribe the extracted RNA after concentration determination. Finally, real-time PCR (Bio-Rad iQ5 real-time PCR detection system) was performed using a mixture of iQ5 SYBR Green I supermix, cDNA templates and each forward and reverse primers as follows: 40 cycles of PCR (95°C for 20 s, 55°C for 10 s, and 72°C for 20 s) after initial denaturation step of 30 s at 95 °C. Alkaline phosphatase (ALP) and osteocalcin (OCN) were evaluated and normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers sequences for the real-time PCR are: ALP, 5’-AACGGCGACAAACAATCATACTACA-3’ and 5’-TGTCCCATCCCCAGGCTGT-3’; OCN, 5’-GTTGCGAACCAGCCAGCAACCA-3’ and 5’-AGTTAGGCCCGGACTTATCTA-3’; and GAPDH, 5’-GCCGCAGCTAGCCGACT-3’ and 5’-ATGCCGTGTCAAGCGGCGCA-3’. Forward and reverse, respectively. Quantification of gene expression was based on the CT (threshold cycle) value of each measurement and presented as the average and standard deviation of three replicates.

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![Fig. 3. Gaussian–Lorentzian fitted Raman spectrum of DLC film deposited by PIII&D on PEEK.](image)

![Fig. 4. FTIR spectra of DLC/PEEK and PEEK control.](image)

![Fig. 5. AFM images of (a) PEEK control and (b) DLC/PEEK.](image)
3. Results

The XPS elemental depth profile acquired from the DLC/PEEK sample is depicted in Fig. 1. The oxygen signal on the surface results from surface contamination and disappears with sputtering, whereas the carbon concentration is 99.7% throughout DLC film except near the surface. As shown in the square frames, the carbon concentration decreases gradually as the oxygen content increases, suggesting a transition region between the DLC film and PEEK substrate. The gradual structural change which indicates a gradual interlayer can serve as the buffer layer to reduce the effects caused by the mismatch in the mechanical properties between DLC and PEEK thereby making DLC deposition on PEEK successful.

As shown in Fig. 2, the C1s core level spectra show broad peaks on the DLC surface (sputtering time of 3 min) and in the PEEK substrate (sputtering time of 120 min). The C1s peak of DLC in Fig. 2(a) can be resolved into two sub-peaks corresponding to Csp³-C-H and Csp², respectively. The C1s spectrum of PEEK in Fig. 2(b) reveals the formation of C=C-C, C=O and C=O. Fig. 2(c) illustrates the shift of O1s which agrees with the structural change disclosed by C1s. While the symmetrical oxygen peak on the sample surface suggests surface contamination, the asymmetrical peak in PEEK can be deconvoluted into two sub-peaks indicative of C=O and C=O.

Fig. 3 displays the Raman spectrum of the DLC/PEEK which can be deconvoluted into two sub-peaks: the G band at 1558.93 cm⁻¹ and D band at approximately 1354.63 cm⁻¹ with the I(D)/I(G) ratio of 1.2. This is a typical spectrum of the DLC structure. The ATR FTIR spectra acquired from the DLC/PEEK and PEEK control are displayed in Fig. 4. Because the DLC film is relatively thin, the DLC/PEEK yields a similar IR response as the PEEK control at 1735 cm⁻¹ showing the

![Fig. 6. (a) Nanohardness and (b) Elastic moduli of DLC/PEEK and PEEK control as a function of indentation depth. Comparison of load–displacement curves of DLC/PEEK and PEEK control at the peak indentation load at: (c) 1300 µN and (d) 3000 µN.](image)

![Fig. 7. Water and ethylene glycol contact angles measured on PEEK control and DLC/PEEK.](image)
strong vibration of C=O. However, in the ATR spectrum of DLC/PEEK, the peaks corresponding to the benzene vibration in PEEK disappear and they are replaced by the alkene C=C peak at 1670 cm\(^{-1}\). This change can be attributed to the DLC film and it is consistent with Raman and XPS data.

The AFM images representing a scanned range of 1 μm are shown in Fig. 5. Significant differences can be observed. The surface of PEEK [Fig. 5(a)] is completely covered by dense buds arising from the graphitic clusters shown in Fig. 5(b). The root-mean-square (RMS) roughness values increase from 3.42 nm (PEEK) to 5.42 nm (DLC/PEEK).

The nanohardness and elastic modulus values of the DLC/PEEK and PEEK control as a function of the indentation depths from 50 to 900 nm are plotted in Fig. 6(a) and (b), respectively. The surface hardness increases from 0.2 GPa to 1.9 GPa while the surface elastic modulus also changes from 5 GPa to 16 GPa after DLC deposition. The higher elastic modulus suggests plastic resistance which is confirmed by the load-displacement curves acquired at loadings of 1300 μN and 3000 μN as shown in Fig. 6(c) and (d). At the same load, the DLC/PEEK sample shows a smaller indentation depth and larger elastic recovery than the PEEK control. It should be noted that there is a step (marked by arrows) during the DLC/PEEK loading. This is generally indicative of ring-like through-thickness cracking. However, continuous curves are observed during the unloading stage and no severe spalling or failure occurs during the loading process.

The contact angles using water and ethylene glycol as the media are shown in Fig. 7. The contact angles measured on the DLC/PEEK are much lower than those on the PEEK control, implying that the DLC/PEEK sample has better wetting properties.

The time-dependent cell viability data are presented as a histogram in Fig. 8. The data reveal that both the cell attachment and subsequent proliferation are improved after DLC deposition. In particular, the absorbance value obtained from the PEEK control after 6 h of incubation is slightly lower than that on the DLC/PEEK sample, although the difference is not significant. However, when the incubation time is up to 3 or 6 days, the values measured from the DLC/PEEK sample are much higher than that on the PEEK control. The SEM images of osteoblasts seeded on both specimens for 6 h are depicted in Fig. 9. The attached cells on the PEEK sample have a spindle-shaped morphology but the osteoblast morphology on the DLC/PEEK sample is more extended and flatter. As shown in Fig. 9(b), protruding cell nuclei (marked by purple arrows) surrounded by cytoplasm can be discerned and so the spread of osteoblasts on DLC/PEEK is evidently better.

The genes expressions of rat calvaria osteoblasts on both DLC/PEEK and PEEK relevant to the culture duration (3, 6 and 12 days) are quantified by real-time PCR and presented as histograms in Fig. 10. It is obvious that the gene expressions increase gradually with culture time. Furthermore, the results indicate that the DLC film promotes the ALP and OCN expressions of the seeded osteoblasts. At all 3 time points, the osteoblasts on the DLC surface exhibit higher ALP expressions than those on the PEEK control. As the culture time increases, the difference in ALP levels between the two samples becomes more significant. On the other hand, no OCN expression of osteoblasts is detected during the initial 3 days of culture. After 6 days of incubation, the OCN expressions emerge and the levels of OCN on both samples are similar. When the incubation time is increased to 12 days, the OCN expression of osteoblasts is much higher on the DLC/PEEK than PEEK control.

4. Discussion

Titanium and its alloys are one of the most common biomaterials used in bone and joint replacements. However, as
discussed previously, the relatively high elastic modulus of Ti prostheses inevitably leads to resorption of adjacent bones due to stress shielding. In this respect, PEEK which is also non-toxic offers many advantages such as an elastic modulus more similar to that of cortical bone compared to metal alloys. Nevertheless, the biocompatibility of PEEK is not good enough and the elastic difference between pristine PEEK and bones is still substantial. In order to tackle this problem, we propose the use of DLC. As far as we know, amorphous carbon films have not been fabricated on PEEK by plasma immersion ion implantation and deposition (PIII&D) before. As shown in Figs. 1–4, our efforts have been successful. It is important to note that the surface roughness value increases by over 50%, and Fig. 7 discloses that the surface hydrophilicity is improved after DLC deposition. The improved hydrophilicity is believed to stem from the change in the surface chemistry and morphology subsequently affecting the difference in the cell response observed in this study.

To evaluate the biocompatibility, the in vitro osteoblast behavior on the DLC/PEEK and PEEK control is studied and compared. Initially, in order to avoid the abnormal phenotype of osteosarcoma cells, hFOB 1.19, a human fetal-osteoblast cell line transfected with a gene coding for a temperature-sensitive mutant (tsAS8) of the SV40 large T antigen [28] is chosen for the tests of osteoblast attachment and subsequent proliferation. The 6 h cell viability measurements do not reveal a significant difference between the DLC/PEEK and PEEK control. However, as shown in Fig. 9, the attached osteoblasts on the DLC surface exhibit a more spreading morphology. It is well known that a sequential process comprising proliferation, ECM deposition, differentiation, and maturation takes place following the initial attachment of osteoblasts on the materials [29]. Osteoblasts adhesion and spreading can greatly influence the subsequent cell performance on the implants. According to Figs. 8 and 9, osteoblast attachment on the DLC/PEEK sample is superior to that on the PEEK control. Fig. 8 also indicates that subsequent osteoblast proliferation is better on the former.

It is noted here that the applications of cell lines are limited by their inability to differentiate. Thus, rat calvaria osteoblasts obtained from calvaria of neonatal rats are used to substitute for hFOB 1.19 cells in the examination of osteoblast differentiation. Alkaline phosphatase (ALP) and osteocalcin (OCN) gene expressions of rat calvaria osteoblasts are quantified by real-time PCR. As one of the chosen proteins for gene quantification, ALP is a marker for early differentiation of osteoblasts. It regulates inorganic phosphate metabolism via hydrolyzing phosphate esters [30,31] and functions as a plasma membrane transporter for inorganic phosphates [32]. OCN is a specific protein only expressed by mature osteoblasts during the post-proliferative period [33–36]. It controls the nucleation and size of the hydroxyapatite crystals in the ECM of bone tissues and regulates bone crystal growth [37]. OCN is the most specific protein in osteoblast differentiation and mineralization among all [38]. Fig. 10 indicates that both the ALP and OCN expressions of osteoblasts on the DLC/PEEK sample are higher than those on the PEEK control incubation time up to 12 days. Hence, our results provide unequivocal proof that the DLC/PEEK combination benefits the differentiation of osteoblasts.

5. Conclusion

We propose the DLC/PEEK combination to enhance the mechanical and biological properties of PEEK. The DLC film is deposited by plasma immersion ion implantation and deposition (PIII&D) to take advantage of the non-line-of-sight characteristic of the technique. Energetic ion bombardment during DLC deposition creates a gradual transition between the DLC film and PEEK substrate. The DLC/PEEK system is almost isostatic to cortical bone leading to improved stability as bone substitutes. As shown by the biological results, osteoblast attachment, proliferation, and differentiation are superior on DLC/PEEK which is a promising material in orthopedic applications.

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Appendix

Figure with essential color discrimination. Figs. 1–5 and 9 in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi: 10.1016/j.biomaterials.2010.07.054.

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