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The electron beam deposition of titanium on polyetheretherketone (PEEK) and the resulting enhanced biological properties

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ABSTRACT

The surface of polyetheretherketone (PEEK) was coated with a pure titanium (Ti) layer using an electron beam (e-beam) deposition method in order to enhance its biocompatibility and adhesion to bone tissue. The e-beam deposition method was a low-temperature coating process that formed a dense, uniform and well crystallized Ti layer without deteriorating the characteristics of the PEEK implant. The Ti coating layer strongly adhered to the substrate and remarkably enhanced its wettability. The Ti-coated samples were evaluated in terms of their *in vitro* cellular behaviors and *in vivo* osteointegration, and the results were compared to a pure PEEK substrate. The level of proliferation of the cells (MC3T3-E1) was measured using a methoxyphenyl tetrazolium salt (MTS) assay and more than doubled after the Ti coating. The differentiation level of cells was measured using the alkaline phosphatase (ALP) assay and also doubled. Furthermore, the *in vivo* animal tests showed that the Ti-coated PEEK implants had a much higher bone-in-contact (BIC) ratio than the pure PEEK implants. These *in vitro* and *in vivo* results suggested that the e-beam deposited Ti coating significantly improved the potential of PEEK for hard tissue applications.

1. Introduction

Polyetheretherketone (PEEK) is a semi-crystalline thermoplastic that exhibits outstanding mechanical and chemical properties, as well as a high thermal stability. Therefore, PEEK can widely be used in diverse number of fields, such as the aerospace, automotive, and chemical process industries. PEEK, which was approved as a medical grade material by the U.S. FDA in the late 1990s, has recently been studied and used as a substitute for metallic implant materials because of its appropriate biocompatibility and extremely low elastic modulus (3 ~ 4 GPa) [1–5], which reduces the extent of stress shielding that is often observed in titanium-based metallic implants [6,7].

Despite its good mechanical properties, the adhesion of PEEK implants to bone tissue proceeds slowly because of their relatively low biocompatibility [3]. The biocompatibility of the implant is strongly affected by its surface characteristics, including the surface roughness, wettability and chemical composition [8]. Therefore, considerable efforts have focused on modifying the surface of the PEEK implants. A representative example of the surface modifications of the PEEK implant is the introduction of bioactive coating materials using various physical and chemical methods, including ionic plasma deposition (IPD) [9], plasma spray deposition [10] and *in vitro* precipitation [11,12]. This coating layer must be biologically beneficial, physically stable and mechanically adherent to the PEEK substrate. Additionally, the coating process must be carried out at relatively low temperatures in order to avoid thermal degradation of PEEK.

Titanium (Ti) is the most widely used implant material for load-bearing dental and orthopedic applications because of its excellent mechanical and biological properties [13,14]. Especially, in terms of biocompatibility, Ti is only surpassed by bioactive ceramics, such as hydroxyapatite or bioglass, and natural biopolymers, such as collagen or its derivatives. Therefore, Ti is a strong candidate as the coating material for PEEK implants. In this research, the electron beam deposition (e-beam deposition) process was used to coat Ti onto PEEK in order to enhance its biocompatibility. The e-beam deposition method is a versatile coating technique that produces a dense, uniform film on any substrate at a low temperature [15,16]. Even though the Ti was deposited onto PEEK at a relatively low

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temperature, the Ti was fully crystallized without the need for any post-deposition heat treatment.

The physical properties of the Ti coating layer were evaluated in terms of microstructure, crystallinity, wetting angle and bonding strength with respect to the PEEK substrate. The effects of the Ti coating layer on the biological properties of PEEK were assessed through *in vitro* and *in vivo* tests. More specifically, the *in vitro* cellular responses of the samples were evaluated in terms of cell attachment, proliferation, and osteoblastic differentiation. The *in vivo* bone conductivity was examined by measuring the bone-to-implant contact (BIC) ratio using a rabbit tibial defect model for a period of 4 weeks.

2. Materials and methods

2.1. Electron beam deposition (e-beam deposition)

Disc shaped PEEK (PEEK-OPTIMA®, Invibio, UK) substrates with a diameter of 15 mm and a thickness of 2 mm were prepared, polished with a 2000-grit SiC abrasive paper, and then ultrasonically cleaned. Commercially pure Ti plates (Kahee Metal, Korea), with dimensions of 10 mm × 10 mm × 1 mm, were prepared as a target material, ground with a 220-grit SiC abrasive paper, and then ultrasonically cleaned. All of the ultrasonic cleaning processes were successively conducted for 3 min each in acetone, ethanol and distilled water. A thin Ti film was deposited onto the PEEK surface using an e-beam evaporator (EVACO-EB800R, DR Vacuum Inc., Korea). The prepared substrate was mounted on a rotating holder in a vacuum chamber and cleaned with an Ar ion beam with a voltage of 90 V and a current of 1.5 A for 20 min before coating. Then the Ti film was coated on the PEEK substrate to a film thickness of 1 μm at a rate of ~0.05 nm/s. The temperatures for the Ar ion beam cleaning and titanium coating processes were approximately 90 °C and 120 °C, respectively. During the coating process, the substrate holder was rotated at 5 rpm to achieve a uniform thickness.

2.2. Characterization

The microstructures and phases of the specimens were evaluated using scanning electron microscopy (SEM; JSM-6360, JEOL, Tokyo, Japan) and X-ray diffraction (XRD; M18XHF-SRA, MacScience Co., Yokohama, Japan) analyses, respectively. The wettability of both of the specimens was evaluated using the sessile drop method. Distilled water drops were applied onto the surface of both specimens and photographed with a CCD camera that was connected to a goniometer (Phoenix 300, Surface Electro Optics Co. Ltd., Korea), so that the contact angle could be calculated. The bond strength between the Ti film and PEEK was determined using tensile tests (Sebastian V, Quad Group, Spokane, WA, USA). During these tests, an aluminum stud was mounted onto the specimen with an epoxy glue and hardened using a heat treatment for 1 h at 150 °C. The load at failure was measured to determine the bond strength.

2.3. Biological properties

2.3.1. In vitro tests

The biological properties were evaluated using both *in vitro* cell tests with an MC3T3-E1 cell line (ATCC, CRL-2593) and *in vivo* animal tests. For the *in vitro* cell tests, the specimens were sterilized overnight using UV irradiation. The pre-incubated cell line was placed onto the specimens at densities of 5×10^4, 2×10^4 and 1.5×10^4 cells/cm^2 for the cell attachment, proliferation and differentiation tests, respectively, and then the cells were cultured in a humidified incubator with 5% CO_2 at 37 °C. An alpha-minimum essential medium (α-MEM, Welgene Co., Ltd., Korea) that was supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin was used as the culturing medium, and 10 mM β-GP and 50 μg/ml ascorbic acid were added for the alkaline phosphatase (ALP) test. The attached cells were observed using confocal laser scanning microscopy (CLSM, Zeiss-LSM510, Carl Zeiss Inc., NY, USA). After 3 h of culturing on the specimens, the cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min, washed in PBS, and permeabilized with 0.1% Trion X-100 in PBS for 5 min. Then the cells were rewashed in PBS and stained with fluorescent phalloidin for 20 min. The proliferation behavior of the cells was determined over a 5-day period using the MTS (methoxyphenyl tetrazolium salt) method. The quantity of the formazan product was directly proportional to the number of living cells and was quantified with the absorbance measurements that were taken at 490 nm using a micro-reader (Biorad, Model 550, USA). The degree of differentiation of the cells was assessed by measuring the ALP activity using p-nitrophenyl phosphate (pNPP) (Sigma–Aldrich, UK). During this test, pNPP was converted into p-nitrophenol (pNP) in the presence of ALP at a rate that was proportional to the ALP activity. The production of pNP was determined using the absorbance that was measured at 405 nm using a micro-reader after 7 and 14 days of incubation. The experimental data for the biological tests were expressed as means ± standard deviations (SD) for $n = 3$.

2.3.2. In vivo tests

The *in vivo* animal tests were carried out on five male New Zealand white rabbits (12 weeks, average weight 3 kg). During the *in vivo* tests, PEEK screws with a diameter of 3.4 mm, thread length of 4.5 mm and total length of 6 mm were prepared. Only one side of the PEEK screw was coated with a 1 μm Ti film in order to compare the osteointegration of the two materials in the same defect. A combination of 1.5 cc of 2% Xylazine HCl (Rompun, Bayer Korea, Korea) and 0.5 cc of Tiletamine HCl (Zoletil, Virbac lab, France) was used as the general anesthesia, and Lidocaine (Yuhan Corporation, Korea) with 1:100,000 epinephrine was injected as the local anesthesia. Tibial defects, with a diameter of 3.4 mm, were created on each of the hind legs using a hand piece drill. The half-coated PEEK screws were implanted into both of the defects for each of the five rabbits. After surgery, the

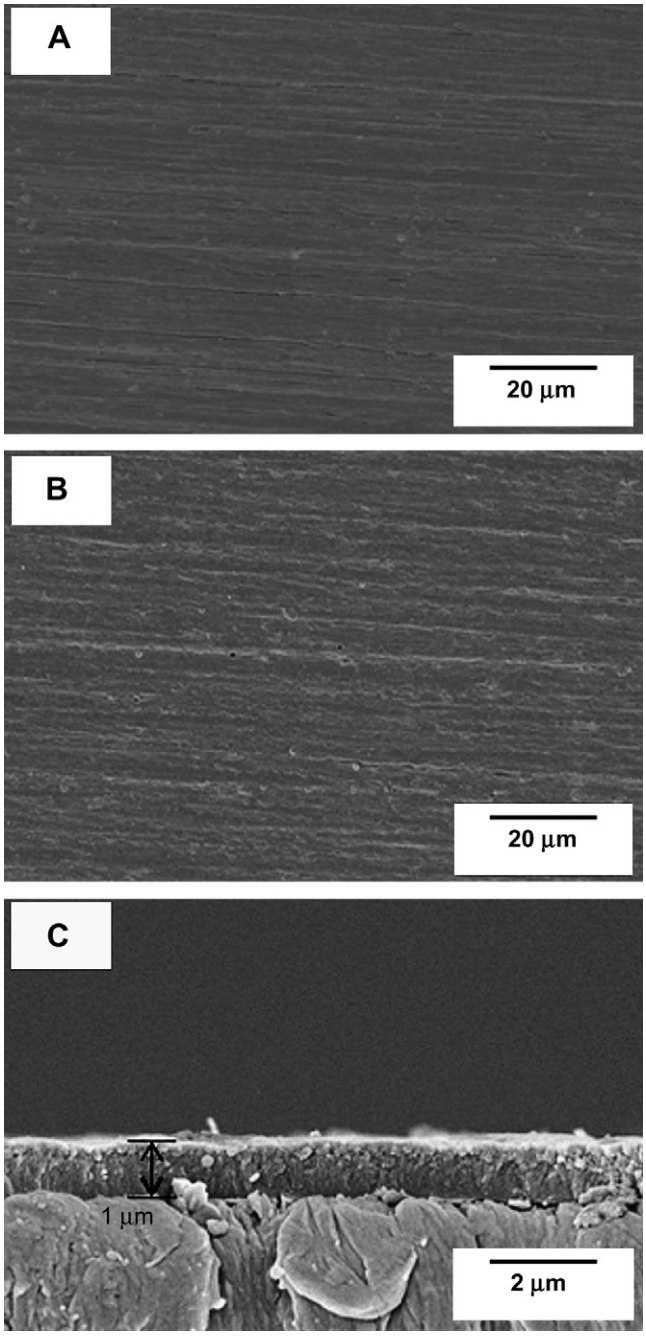

Fig. 1. Scanning electron microscopy (SEM) images of the surface of (A) as-machined and (B) Ti-coated PEEK and (C) the cross-section of Ti-coated PEEK.