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Microwave assisted coating of bioactive amorphous magnesium phosphate (AMP) on polyetheretherketone (PEEK)

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ABSTRACT

Polyetheretherketone (PEEK) with great thermal and chemical stability, desirable mechanical properties and promising biocompatibility is being widely used as orthopedic and dental implant materials. However, the bioinert surface of PEEK can hinder direct osseointegration between the host tissue and PEEK based implants. The important signatures of this paper are as follows. First, we report for the formation of osseointegrable amorphous magnesium phosphate (AMP) coating on PEEK surface using microwave energy. Second, coatings consist of nano-sized AMP particles with a stacked thickness of 800 nm. Third, coatings enhance bioactivity in-vitro and induce significantly high amount of bone-like apatite coating, when soaked in simulated body fluid (SBF). Fourth, the as-deposited AMP coatings present no cytotoxicity effects and are beneficial for cell adhesion at early stage. Finally, the high levels of expression of *osteocalcin* (OCN) in cells cultured on AMP coated PEEK samples indicate that AMP coatings can promote new bone formation and hence osseointegration.

1. Introduction

Since late 1990s, Polyetheretherketone ($(-C_6H_4-O-C_6H_4-O-C_6H_4-CO-)_n$; PEEK) had emerged as a promising implant biomaterial for trauma, orthopedic and spine operations, due to its outstanding chemical and wear resistance, thermoplastic property and non-toxicity. In addition, mechanical properties of PEEK, such as elastic modulus, tensile strength, stiffness and fatigue are comparable to those of cortical bones [1–4]. On account of its high thermal, chemical, radiation and mechanical stability, PEEK can be effectively sterilized and shaped to desirable structure [1]. Moreover, the radiolucency of PEEK can facilitate the clear assessment of status of bony fusion [1,2]. The surfaces of PEEK are biologically inert, thus presenting limited ability to directly bond with the surrounding tissues. The bioinert nature of PEEK significantly hinders its clinical applications in situations where osseointegration is critical. One strategy to address this drawback is to incorporate the hydroxyapatite (HA) as a filler in PEEK matrix or deposit HA layer on PEEK surface to promote the osseointegration of PEEK [5–13]. The similar strategy has also attracted thriving interest in the fabrication of multifunctional hybrid biomaterials composed of degradable biopolymers and inorganic materials for biological applications [14–16], such as gene and drug delivery [17,18], biosensors [19–25], catalysts, therapy [16,19,26] and antimicrobial [18,27].

Apatitic phases is a group of bioactive ceramics, have been extensively investigated as the coating materials on metallic implants, because of its great similarity to bone minerals and excellent osteoconductive properties [28,29]. Alternatively, various techniques have been proposed and explored to deposit HA or other forms of calcium phosphate coatings on PEEK surface, such as thermal plasma spray [5], cold spray [8], spin coating [11], chemical deposition [30], sputtering [31], ion beam assisted deposition [32], biomimetic [33] and microwave assisted coating [34]. It's noteworthy that the PEEK surface can also be modified by chemical and physical methods, such as sulfonation and plasma treatment, to enhance apatite deposition in physiological condition [35,36].

Recently, our group developed a novel microwave assisted coating process, which has been demonstrated to be a promising method to perform rapid surface modifications of various bioimplants [34,37–39]. It is known that thermal plasma spray involves high temperature, which can damage polymer substrate [31]. In comparison, the physical deposition techniques like spray and sputtering are only applicable to 2-dimentional structure. Although chemical deposition does not have the aforementioned limitations, the process, especially biomimetic technique is fairly time-consuming and susceptible to bacterial contamination. The microwave assisted coating process can deposit uniform bioactive coating on the polymer substrate with complex shape at

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relatively low temperatures, and within a short time-scale.

Of late, compounds in the $MgO-P_2O_5$ binary system are getting a great deal of attention in potential applications in orthopedics and dentistry due to their optimal combination of biocompatibility and biodegradability. A fundamental reason for their success is that magnesium is the fourth most abundant element within the human body. In spite of that, research activities on magnesium phosphates are at still at a relatively smaller scale, as compared to their counterparts, calcium phosphates. Among magnesium phosphates, two crystalline compounds, such as struvite ($NH_4MgPO_4{\cdot}6H_2O$) and newberyite ($MgHPO_4{\cdot}3H_2O$) are front runners in orthopedic applications. Their main applications have been in developing non-exothermic, self-setting, orthopedic cement compositions with high compressive strength, biocompatibility and biodegradability [40–44]. As opposed to crystalline magnesium phosphates, our group has directed concentrated effort in exploring amorphous magnesium phosphate (AMP) [45–48]. We reported that the amorphous phosphate nano-particles elicit high levels of cellular response and gene expression [46]. However, the variety of studies regarding the synthesis and further application of magnesium phosphate is still limited. To best of our knowledge, this is the first attempt to use amorphous magnesium phosphate (AMP) layer as the bioactive interface between polymer implant and host tissue. The objectives of this study are 1) synthesis of AMP coating on PEEK substrate via microwave assisted coating process, 2) investigate the in-vitro behavior such as bioactivity and cytocompatibility of the AMP coated PEEK.

2. Experimental

2.1. Material preparation

Medical grade PEEK disks (Orchid implant solutions, Michigan) with a dimension of Ø10 × 2 mm^3 were employed in this study. All the samples were ground up to #1200 SiC abrasive paper to ensure the smooth surfaces and ultrasonically cleaned in acetone for 10 min to remove the attached debris. Then the PEEK disks were divided into two groups. One group was incubated in 10 M NaOH for 48 h. The other batch of PEEK samples was immersed in concentrated sulfuric acid (95–98 wt%, Fihser Sci) for 10 min to produce a uniform porous structure and rinsed with deionized (DI) water subsequently. After the pre-treatment, the first batch of PEEK samples was denoted as PEEK-OH and the second batch was denoted as PEEK-S.

2.2. Coating preparation

The coating bath was prepared by dissolving 2.033 g $MgCl_2$ and 1.1998 g NaH_2PO_4 in 200 ml DI water. 1 M NaOH solution was used to adjust the pH value of coating solution to 6.8. The pretreated PEEK disks were placed in a 200 ml pyrex beaker, which was filled with 100 ml coating solution. Then the beaker with PEEK samples and coating bath were irradiated in a microwave oven (Panasonic) at 1200 W power for 5 min. To ensure the uniformity of deposited layer, the above coating procedure was replicated. The coated PEEK samples were subsequently taken out from coating bath and rinsed with DI water followed by air drying.

2.3. Characterization and bioactivity evaluation

The contact angle measurements were carried out using a contact angle meter (Model CAM-MTCRO, Tantec) to evaluate the wettability of samples. The surface morphologies and elemental compositions of the pretreated and AMP coated specimens were analyzed using scanning electron microscopy (SEM, S4800, Hitachi) coupled with an energy dispersive X-ray spectroscopy (EDS, Oxford INCA). Elemental analyses were performed at an accelerating voltage of 20KV with the working distance of 15 mm. Phase compositions of the samples were identified by X-ray diffraction (XRD, Ultima III, Rigaku) with monochromated Cu Kα radiation (44KV, 40 mA) over a 2θ range of 10–45°. Functional groups present in as-deposited coatings were characterized by a Fourier Transform Infrared Spectroscopy (FTIR, UMA-600 Microscope, Varian Excalibur Series) using an ATR diamond crystal for 256 scans at the range between 4000 and 700 cm^{-1} with a resolution of 1 cm^{-1}. The transmission electron microscopy (TEM, HD-2300, Hitachi, USA) was employed to investigate the microstructure of as-deposited AMP coatings.

Table 1
Ion concentrations of the SBF and human blood plasma.

Ion	Concentration (mM)	
	Human blood plasma	Simulated body fluid (pH 7.4)
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	125.0
HCO_3^-	27.0	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

To evaluate the in vitro bioactivity, both coated and uncoated samples were immersed in simulated body fluid (t-SBF) at 37 °C for 7 days. The ion concentrations of t-SBF that better mimics human blood plasma are listed in Table 1 [49]. In addition, the supersaturation conditions were maintained by replenishing the t-SBF solution every other day. After immersion, the samples were rinsed with deionized water and dried in air for further characterization of SEM and EDS.

2.4. Cell viability assay

MC3T3-E1 (CRL-2593™, ATCC, Manassas, VA, USA) preosteoblast cells were employed to study the effects of AMP coatings on preosteoblast proliferation and differentiation. The preosteoblast cells were initially cultured in alpha minimum essential medium (α-MEM, Thermo Scientific HyClone), augmented with 10% Fetal Bovine Serum (FBS, Thermo Scientific HyClone) at 37 °C in a humidified atmosphere of 5% CO_2. The culture medium was replenished every other day until the cell reached a confluence of 90%. α-MEM culture medium acted as a control medium. For cytotoxicity test, MC3T3-E1 cells were seeded to the sterilized samples in a 24-wells cell culture plate (Flacon™ BD Biosciences, USA) at a density of 10,000/well for 24 h to allow attachment. After further incubation for 3 days, the cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) for 4 h. The formazan precipitate was dissolved in dimethyl sulfoxide (DMSO) and measured using a microplate reader at a wavelength of 570 nm to assess live cells.

To evaluate the expression of osteogenesis-related genes, some cells were subject to RNA isolation after 7 days incubation by the TRIzol regent (Invitrogen, Carlsbad, CA, USA), and the total RNA was reversely transcribed to complementary DNA (cDNA) using M-MLV reverse transcriptase (Promega, Madison, WI, USA). The expressions of the alkaline phosphatase (ALP) and osteocalcin (OPN) were quantified using real-time polymerase chain reaction (PCR) with SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA) and a 2-step amplification program (30 s at 95 °C and 60 s at 62 °C) performed on an Eppendorf Realplex thermal cycler. The relative mRNA expression level of each gene was determined by the CT (cycle threshold) values with normalization to the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The forward and reverse primers for targeted genes are listed as follows: osteocalcin (OCN; forward 5′-GCAATAAG GTAGTGAACAGACTCC-3′ and reverse 5′-CTTTGTAGGCGGTCTTCA AGC-3′) and alkaline phosphatase (ALP; forward 5′-ATCTTTGGTCTGG