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Porous titanium layer co-immobilized with bone morphogenetic protein-2 and vancomycin for biofunctionalization of ultra high molecular weight polyethylene

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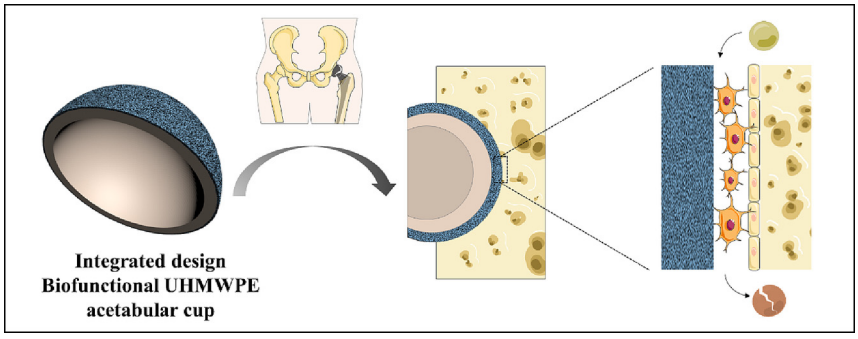
HIGHLIGHTS

- Porous titanium-coated UHMWPE for joint prostheses were developed via hot-pressing.
- BMP-2 and vancomycin were co-functionalized on Ti-coated UHMWPE.
- Biofunctionalized UHMWPE promoted cell adhesion and inhibited biofilm formation.
- Surface bio-functionalized UHMWPE acetabular cup promoted bone integration *in vivo*.

GRAPHICAL ABSTRACT



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ABSTRACT

Ultra high molecular weight polyethylene (UHMWPE), a common material for artificial joint linings, lacks appropriate bioactivity and antibacterial property, which could lead to prosthetic loosening and infections after surgery. Herein, a biofunctionalized UHMWPE material was developed by constructing a firmly bonded porous titanium layer on UHMWPE surface through hot-pressing. The micron-scale titanium surface was then activated by tannic acid treatment, and co-immobilized with Bone Morphogenetic Protein-2 and vancomycin. Scanning electron microscopy, mechanical testing, contact angle measurement, X-ray photoelectron spectroscopy analysis, and infrared spectrometry confirmed the successful construction of the coatings on UHMWPE without causing obvious oxidative degradation. The biofunctionalized UHMWPE surface significantly promoted cell adhesion and proliferation, and inhibited bacterial formation *in vitro* and *in vivo*. A preliminary *in vivo* study of canine hip replacement showed that the UHMWPE acetabular cup with the coating had good biocompatibility and promoted new bone formation and integration. These findings support the use of biofunctionalized UHMWPE as promising materials for artificial joint prostheses.

1. Introduction

With the accelerated ageing of the population, the incidence of joint disease and the demand for artificial joint replacement

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surgery are rising [1]. Total joint replacement is the only effective treatment option for osteoarthrosis and severe injuries [2]. Ultra high molecular weight polyethylene (UHMWPE) is a common material used for artificial joint component due to its light weight, outstanding water resistance, excellent mechanical properties, good biochemical resistance, and high wear resistance [3]. However, because UHMWPE is a bioinert material, it cannot be directly fixed with bone tissue. As a result, titanium acetabular cups are traditionally used to strengthen the interaction between joint prosthesis and surrounding tissue. However, the modulus of the metal cup (90–110 GPa) is substantially higher than that of human bone tissue (0.3–30 GPa), inevitably resulting in stress shielding and aseptic loosening in long-term use, leading to dislocation of the prosthesis [4], which would result in secondary surgical revision. To address this problem, developing bioactive, low-modulus UHMWPE acetabular cups without the use of metal components is a potential solution. To do this, the UHMWPE material needs to be modified with a biofunctional coating to promote cell adhesion and proliferation on the surface, thereby improving the integration of the joint prosthesis with the surrounding tissues. In addition, the issue of infection following joint prosthesis installation should not be overlooked. It is estimated that 28 $\sim$ 30% of implant failures are caused by aseptic loosening, whereas 15 $\sim$ 20% are caused by infection [5]. In the event of infection, the patient will suffer immense pain. Therefore, it is also necessary to develop prostheses with antibacterial features.

Biomimetic highly porous UHMWPE/hydroxyapatite (HA) scaffolds loaded with Bone Morphogenetic Protein-2 (BMP-2) have been produced for reconstruction of nonload-bearing bones [6,7]. However, because the scaffold with a high content of HA is highly porous, it may not be suited for load-bearing applications such as artificial joints. On the other hand, the surface hydrophilicity and topography are primarily determined by the surface chemistry and roughness of the biomaterial, and they affect the cytocompatibility of the implant but not its mechanical properties [8]. Various techniques, such as magnetron sputtering [9], plasma spraying [10], glow treatment [11], and chemical vapour deposition [12] have been applied to improve the biological activity of UHMWPE. However, these modification techniques were relatively harsh and difficult to implement. Coatings for alteration of UHMWPE surface properties have been developed in recent years, primarily using wet chemical process. For instance, Ai et al. [13] enhanced the biofunctionality of UHMWPE by constructing a silk fibroin coating loaded with vascular endothelial growth factors (VEGF) on the surface. Chen et al. [14] reported a chemical grafting method to graft polyvinyl alcohol hydrogel on UHMWPE to improve surface hydrophilicity and lubricity. In addition, antimicrobial agents such as antibiotics and metal ions or nanoparticles have been introduced into UHMWPE to improve its antimicrobial properties. Gentamicin-loaded chitosan was impregnated in the solvent-etched and lyophilized porous surface of carbon nanotube-reinforced UHMWPE to obtain a prolonged release of the antibiotics and eradicate the surrounding bacteria [15]. Different morphologies of antibacterial zinc oxide were blended with UHMWPE, and the effect of zinc oxide morphology on the antibacterial properties of UHMWPE was investigated [16]. In clinic practice, aseptic loosening of joint implants and post-operative infections usually coexist, therefore a solution to improve both the cell affinity and antimicrobial properties of UHMWPE is required. However, to the best of our knowledge, few studies have been conducted to simultaneously increase the bioactivity and antimicrobial function of UHMWPE.

Porous titanium with interconnective macro/microporous structure has been reported to have good biocompatibility and can stimulate osteogenic induction [17]. Titanium-modified UHMWPE samples have been prepared by magnetron sputtering, and allowed for high cell proliferation and alkaline phosphatase (ALP) expression [18]. However, the experimental conditions of magnetron sputtering, on the other hand, were harsh and not favorable to manipulation. In this study, a convenient hot-pressing process was employed to create an UHMWPE/porous titanium hybrid layer on UHMWPE surface, thereby improving its bioactivity. The titanium layer was activated by tannic acid (TA) treatment, and BMP-2 and vancomycin were co-immobilized onto the TA-treated porous titanium surface to further enhance the osteogenic property and antibacterial function of UHMWPE. The surface morphology and hydrophilicity of the coating were characterized. The bonding strength of the porous titanium layer to the UHMWPE substrate was measured. Finally, the antibacterial and osteogenic properties of the modified UHMWPE were evaluated *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Ultra high molecular weight polyethylene (UHMWPE) block was prepared as the procedure described in the previous study [19], and processed into disks with 10 cm in diameter and 5 mm in thickness. Titanium powders (catalogue number: TA1) were bought from Avimetal Powder Metallurgy Technology (Beijing, China), and filtered using a 150 mesh sieve to collect the powders with a diameter of $\sim$100–150 μm before use. Nitric acid, sodium hydroxide, tannic acid (TA), tris(hydroxymethyl)aminomethane, and vancomycin hydrochloride were obtained from Macklin (Shanghai, China). Bone Morphogenetic Protein-2 (BMP-2) was purchased from Solarbio (Beijing, China). *Staphylococcus aureus* (*S. aureus*) 5622 was provided by the First Affiliated Hospital of Ningbo University. Mouse embryonic osteoblast precursor cells (MC3T3-E1) were obtained from Fenghui Biotechnology (Hunan, China). Cell Counting Kit-8 (CCK-8) was purchased from Beyotime (Shanghai, China).

2.2. Preparation of porous titanium powder

Porous titanium powder was prepared using the alkali and heat treatment as reported in the previous study [20]. Briefly, 8 g titanium powders were immersed in 40 mL of 6.8% of nitric acid, and ultrasound for 10 min to clean the surface of the titanium powder. The titanium powders were washed repeatedly using deionized water until the solvent become neutral, and then immersed in 1.0 M of sodium hydroxide solution, incubated at 60 °C for 3, 6, 12, and 24 h, respectively. The samples were washed using deionized water and subsequently heated in an electrical furnace at a heating rate of 10 °C /min and kept at 600 °C for 1 h. X-ray diffraction (XRD) patterns of titanium powders were recorded using an X-ray diffractometer (D8 Advance) at 40 kV and 25 mA with a scanning angle 2 theta ranging from 20° to 80°, a step-scan interval of 0.02° and a scanning speed of 4° min^{-1}. The surface morphology of the titanium powders was observed using scanning electron microscopy (SEM, Regulus 8230, Hitachi). ImageJ software (Version 1.52p) was used to measure the particle size and pore size of titanium powder after the alkali and heat treatment.

2.3. Hot-pressing titanium on UHMWPE

Porous titanium powder was impressed onto the surface of UHMWPE through a hot-pressing process. The UHMWPE disk with porous titanium powders spreading evenly on its upper surface (5 g of titanium powders over 78.5 cm^2 of the disk surface) was sandwiched in a custom-made mold and placed in a plate vulcan-

izing press machine (DR-50, Derui Instrument Equipment, Yangzhou, China). The upper plate contacting the side with porous titanium powder was pre-heated to a predetermined temperature (160 °C to 220 °C), and the pressure between the two plates was maintained at 3 MPa for 15 min to melt and soften the surface of the UHMWPE plate. The porous titanium powders were impressed into the UHMWPE surface under the pressure and heating. The sample after the hot-pressing process was immediately transported to another plate vulcanizing press machine at room temperature for cold-pressing with the pressure at 3 MPa for 15 min for annealing. All the samples were taken out and cleaned in an ultrasonic bath for 30 min to remove any loosely attached titanium powder on the surface. The samples were cut into small disks with 7 mm in diameter using a punch. The UHMWPE plate coated with porous titanium powder was denoted as UHMWPE-Ti. Porous titanium-coated UHMWPE acetabular cups was prepared in a similar manner as described above using the hot-pressing process with a customized mold (Fig. S1), and the details of the procedure are given in the Supporting Information.

2.4. Surface modification of UHMWPE-Ti

The UHMWPE-Ti disk (7 mm in diameter) was placed in a 15 mL centrifuge tube containing 10 mL of 2 mg/mL TA solution (10 mM Tris-HCl buffer, pH = 8.1). The tube with the sample was incubated at 37 °C with shaking at 100 rpm for 24 h, washed thoroughly using deionized water, and dried in nitrogen flow. The UHMWPE-Ti disk coated with TA was denoted as UHMWPE-Ti-TA. The UHMWPE-Ti-TA disk was sterilized under UV irradiation for 30 min, and placed in a 48-well plate. Firstly, 10 μg of BMP-2 and 50 mg of vancomycin hydrochloride were dissolved in 1 mL sterile deionized water to obtain the coating solution similar as the previous studies [21,22]. Next, 70 μL of the coating solution was dropped on the surface of the UHMWPE-Ti-TA sample in each well of the 48-well plate. The 48-well plate containing the samples was incubated statically at 4 °C for 24 h. The sample was then washed using sterile deionized water to remove any unbound reagent, and the obtained sample was air dried, and denoted as UHMWPE-Ti-TA-B/V. Immobilization of BMP-2 and vancomycin on titanium-coated UHMWPE acetabular cups was prepared in a similar manner, and the details of the procedure are given in the Supporting Information.

2.5. Surface characterization

Surface properties of the pristine and modified substrates was characterized using contact angle goniometer (DSA25E, KRUSS, Germany), and X-ray photoelectron spectroscopy (XPS, Kratos AXIS ULTRA DLD). The cross-section of UHMWPE was observed using SEM. The possible oxidation of UHMWPE during the hot-pressing process was investigated using infrared spectrometer (Nicolet iS50, Thermo Scientific). The bonding strength of the UHMWPE/-porous titanium hybrid layer to the UHMWPE substrate was measured using a universal testing machine (CMT 5205, MTS, China). The details of the experimental procedure are given in the Supporting Information.

2.6. In vitro antibacterial assay

In vitro antibacterial properties of the samples were investigated using the Zone of Inhibition test and biofilm formation assay. The details of the experimental procedure are given in the Supporting Information.

2.7. In vitro cell assay

UHMWPE disk samples (7 mm in diameter) were placed in each well of a 48-well plate, and sterilized under ultraviolet irradiation for 1 h prior to the assay. MC3T3-E1 cells were cultured in Minimum Essential Medium α (α-MEM, supplemented with 10% fetal bovine serum, 10^5 U/L penicillin and 100 mg/L streptomycin). The cells were suspended in fresh culture medium to a concentration of 10^5 cells/mL, and 0.5 mL of the cell suspension was seeded on the surface of the UHMWPE samples. The plate with the samples was incubated at 37 °C under a humidified atmosphere of 5% CO_2 for 1, 4, or 7 days. The culture medium was replaced every day. Adhesion and growth of the cells on the disk surfaces were characterized by SEM, CCK-8 assay, and alkaline phosphatase (ALP) activity assay as per the manufacturers' instructions.

2.8. In vivo antibacterial study

Female Sprague Dawley (SD) rats (230 ~ 260 g body weight) were obtained from the Vital River Laboratories (Beijing, China) and used for evaluation of the *in vivo* antibacterial effect of the samples. All the procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Biological and Medical Engineering, Guangdong Academy of Sciences. The care and use of the animals conformed to the Guideline for the Care and Use of Laboratory Animals. Six SD rats were divided into two groups (3 rats for each group). A strip incision of 4 cm in length was created on the upper back of each rat. Twenty μL of *S. aureus* bacterial suspension (10^9 CFU/mL) was added to the incision. After 5 min for absorption of the suspension, a sample disk (UHMWPE or UHMWPE-Ti-TA-B/V, 7 mm in diameter and 1.2 mm in thickness) was implanted into the wound. For the UHMWPE-Ti-TA-B/V sample, the coating side contacted the dermis layer. The wound was sutured, and the animal was fed according to the standard protocol. The rats were sacrificed after one week. The tissues around the wound site were harvested, and homogenized for quantification of the viable bacteria using the spreading plate method.

2.9. In vivo acetabular cup implantation study

All procedures of the animal experiment were approved by the Institutional Animal Ethical Committee of Sanitation & Environment Technology Institute of Soochow University Ltd. Three female Labrador dogs (~20 kg body weight) were used and pre-fed for 7 days before implantation, according to the previous study [2]. The UHMWPE acetabular cups with biofunctional coating (25 mm and 27 mm in diameter) were prepared in sterile condition and packaged, and the prosthetic femur stems were sterilized by autoclave. Before surgery, animals were anesthetized by intramuscular injection of Zoletil in combination with Xylazine, and total replacement of unilateral hip joints was performed for all dogs. The operational region of the animal's left leg was shaved and disinfected with betadine solution, using the anterolateral access to the hip joint, and the skin and subcutaneous tissue were incised in a single pass. The femoral head was removed and compared with the canine artificial acetabular cup to select the acetabular cup with the appropriate size for implantation. The soft tissue in the acetabulum was removed, and the acetabular cup was then pressed into the acetabulum. A small amount of bone cement was given to the edge of the cup if direct fixation was difficult. The sterilized artificial femur was inserted into the femoral medullary cavity. The femoral head was repositioned into the acetabular cup, and the wound was closed after the implantation was completed. The animals were kept in separate cages after surgery and treated with intramuscular penicillin for 3 consecutive days. The animals were fed and observed regularly for 3 months before sac-

rifice. The implanted acetabular cup and the underlying tissue were harvested, and fixed in 75% alcohol for 24 h at room temperature, before being subjected to micro-computed tomography (micro-CT) scanning (SkyScan 1176, Bruker). The original data from the micro-CT scanning were managed using the three-dimensional reconstruction software (NRecon Version 1.7.4.6) and analysis software (CT Analyser Version 1.10) to observe the cup-bone interface and surrounding bone trabeculae microstructure.

2.10. Statistical analysis

A one-way analysis of variance (ANOVA) program combined with a student *t*-test was used to evaluate the statistical significance of the variance. Values of $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ were considered statistically significant.

3. Results and discussion

3.1. Preparation and characterization of porous titanium powder

Alkali and heat treatment has been used for modification of titanium powders to obtain porous structures on the surface. The titanium powders were first cleaned by nitric acid, and then subjected to alkali and heat treatment at 60 °C for a determined period. The titanium dioxide layer on titanium surface reacted with hot sodium hydroxide solution to form a sodium titanate gel, and then this gel was dehydrated by heat treatment to form an amorphous sodium titanate layer with a porous structure [23]. This was proved by XRD patterns (Fig. S2), which is consistent with the previous study [4]. Fig. 1 and Fig. S3 showed the surface morphology of titanium powder before and after the alkali and heat treatment. The pristine titanium powder was round and ball-shaped with the diameter of mostly 100–140 μm and a small number of inclusions of small size particles, and the surface was smooth. After alkali and heated treatment, the size of titanium powder basically unchanged, that was because the reaction took place only on the surface of the titanium powder particles and influence negligibly on the particle size. After 3 h of alkali and heat treatment, the surface of the titanium powder became rough. And the surface roughness increased as the treating time increased. After 12 h, a continuous honeycomb-like porous structure appeared on the surface, and the pore size reached to about 125 nm (Fig. S4). Further extending the treating time to 24 h resulted in a macro-porous structure on the surface of titanium powder with a pore size of about 150 nm. These macropores were well interconnected by micropores. In sum, after the alkali and heat treatment, the surface of titanium powder became rough and porous, while the overall spherical structure of the powders was maintained. As a highly porous structure is beneficial to osteogenesis [17], and it could enhance the interconnection with the UHMWPE substrate, titanium powder with a high porosity (alkali and heat treatment for 24 h) was used in the rest of the study for UHMWPE surface modification.

3.2. Surface modification and characterization

Titanium has a high melting point of 1670 °C [24], while the melting point of UHMWPE was about 140 °C [25]. Because of the significant difference in the melting point of the two substrates, hot-pressing technology was used to partially melt the UHMWPE surface and impress the titanium powders to form an UHMWPE/titanium hybrid layer on the UHMWPE substrate (Fig. 2a). A layer of porous titanium powders was first introduced on the surface of UHMWPE (~0.064 g/cm^2). The titanium powder layer was heated to a temperature slightly higher than the melting point of UHMWPE, thereby causing the contacting UHMWPE surface to soften and melted. A constant pressure of 3 MPa was applied on the substrate to impress the titanium powders into the UHMWPE surface. As can be seen from Fig. 2b, the titanium powders were evenly embedded into the plastic surface, forming an UHMWPE/titanium hybrid layer with a thickness of around 200 μm. Since the pressure used (3 MPa) was significantly lower than the compressive yield strength of porous titanium (85 MPa) [26], the morphology and porosity of the embedded titanium powder remained unchanged, this was important for the further modification process and retaining the bioactivity of the titanium layer. Furthermore, energy-dispersive X-ray spectroscopy (EDS) elemental mapping image proved that titanium powder tightly embedded in UHMWPE substrate (Fig. S5). The bonding strength between the titanium layer and the UHMWPE substrate was measured to be about 10.50 MPa in the hot-pressing process at 160 °C or 220 °C. When the titanium powders were embedded into the plastic at 190 °C, the bonding strength increased slightly to 12.86 MPa (Fig. 2c). At 160 °C, due to the heat transferred to the mold and surrounding environment, the temperature at the surface was too low to melt the plastic material for the titanium powders to be embedded. In contrast, temperatures higher than 220 °C were detrimental to the formation of an UHMWPE/titanium hybrid layer. The UHMWPE fluid at high temperatures had a high tendency to flow, and would cause an uneven distribution of titanium powder on the surface. The bonding strength of the titanium layer to the UHMWPE substrate is higher than the reported value of tensile stresses within the acetabulum (0–5 MPa) [27], showing that the titanium-coated UHMWPE could be used in the body. It has been reported that UHMWPE was prone to oxidation at high temperatures and may reduce its mechanical strength and wear resistance [28]. Fourier transform infrared (FTIR) spectroscopy was used to investigate the possible oxidative degradation of the UHMWPE layer in contact with hot-pressed titanium particles, and the oxidation index of UHMWPE before and after hot-pressing at different temperatures was found to be similar without significant differences among the different groups (Fig. S6). The low oxidative effect of the UHMWPE after the hot-pressing process might be due to the short processing time and the relatively low temperature in the hot-pressing process. Since the titanium layer formed the highest bonding strength to the underlying UHMWPE material at 190 °C, as well as the condition did not result in materials oxidation, this temperature was selected in the hot-pressing process in the rest of the study. The excellent bonding strength between the titanium layer and the UHMWPE substrate prevented the titanium powders dislodging from the UHMWPE substrate, thus maintaining a stable structure and biofunction in the artificial joint application.

To further improve the biological activities, the UHMWPE-Ti surface was activated by polyphenol treatment (i.e. TA), and then immobilized with growth factors and antibiotics. Firstly, TA was anchored on the Ti surfaces via direct adsorption [29,30]. Then, BMP-2 and vancomycin were co-immobilized onto the UHMWPE-Ti-TA surface via the hydrogen bonds and Schiff-base reaction/Michael addition between the biomolecules and TA [31]. XPS measurement was used to verify the successful coatings on UHMWPE surfaces (Fig. S7, details were given in Supporting Information). The water contact angle of each surface was recorded (Fig. 2d). The average contact angle on the pristine UHMWPE surface was 103.8°. After coating with a porous titanium layer, the surface contact angle increased to 124.0°. This is because the interconnective micro-structure endowed the surface with high roughness, which rendered it increased hydrophobicity [32]. TA coating on the UHMWPE-Ti surface decreased the contact angle to 73.7°, which is consistent with the previous study that TA coating improved the surface hydrophilicity due to the high amount of

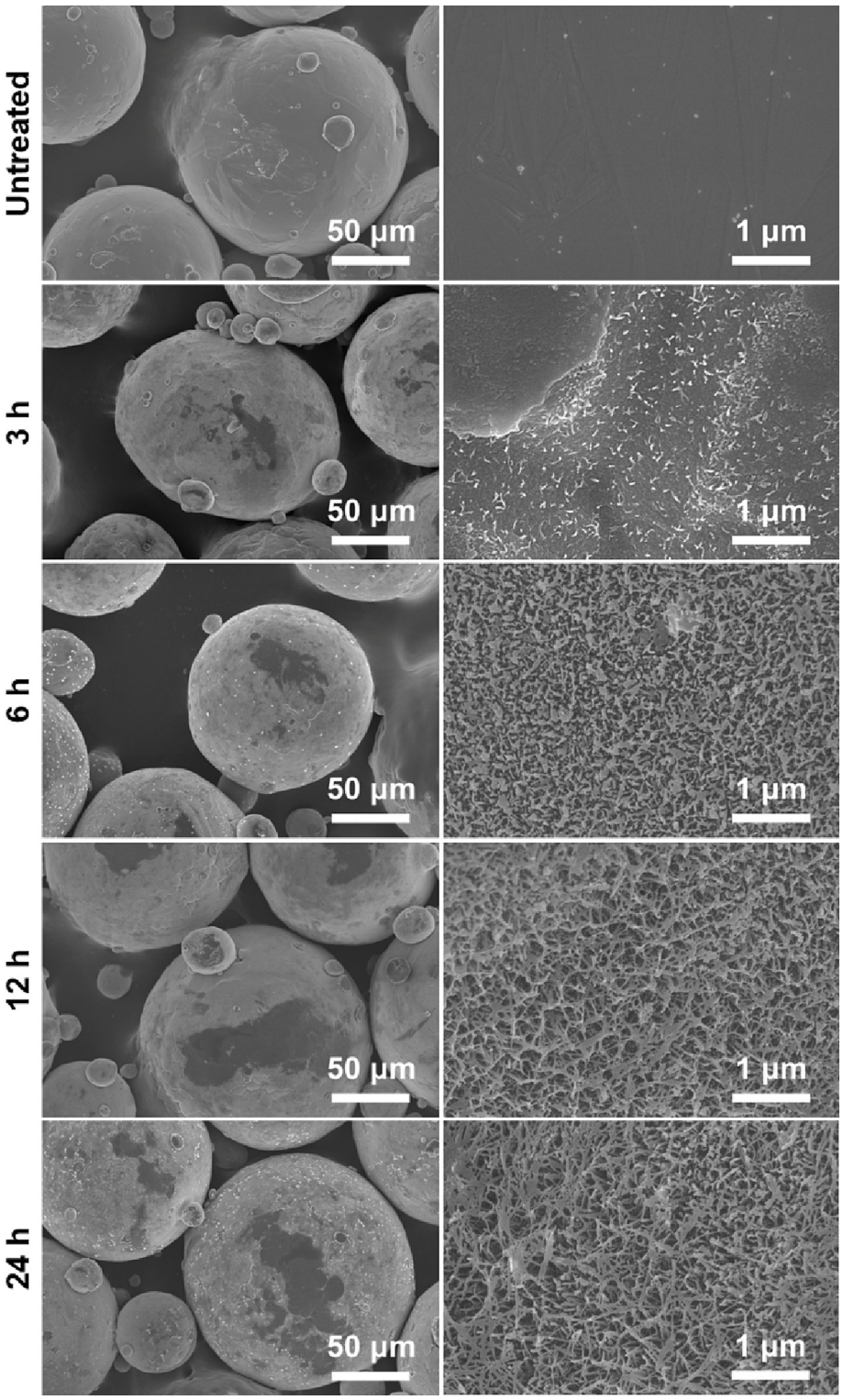

Fig. 1. Surface morphology of titanium powders before and after alkali and heat treatment for 3, 6, 12, and 24 h.

phenolic groups in TA [33]. It is remarkable that the UHMWPE-Ti-TA-B/V surface was fully wetted, confirming the successful graft of biomolecules (i.e. BMP-2 and vancomycin) on the surface.

3.3. In vitro antibacterial activity

Bacterial infection mainly by Gram-positive bacteria like *S. aureus* after joint replacements has been considered one of the most challenging issues, which causes surgical failure, and even severe complications [34]. Vancomycin was commonly used as an antibacterial therapy for osteoarthritis [35]. In this study, vancomycin was immobilized on the UHMWPE surface to endow it with antibacterial property. The antibacterial performance of the modified UHMWPE was investigated by the zone of inhibition test and biofilm formation assay. As shown in Fig. 3a, no zone of inhibition was observed around the pristine UHMWPE, and the

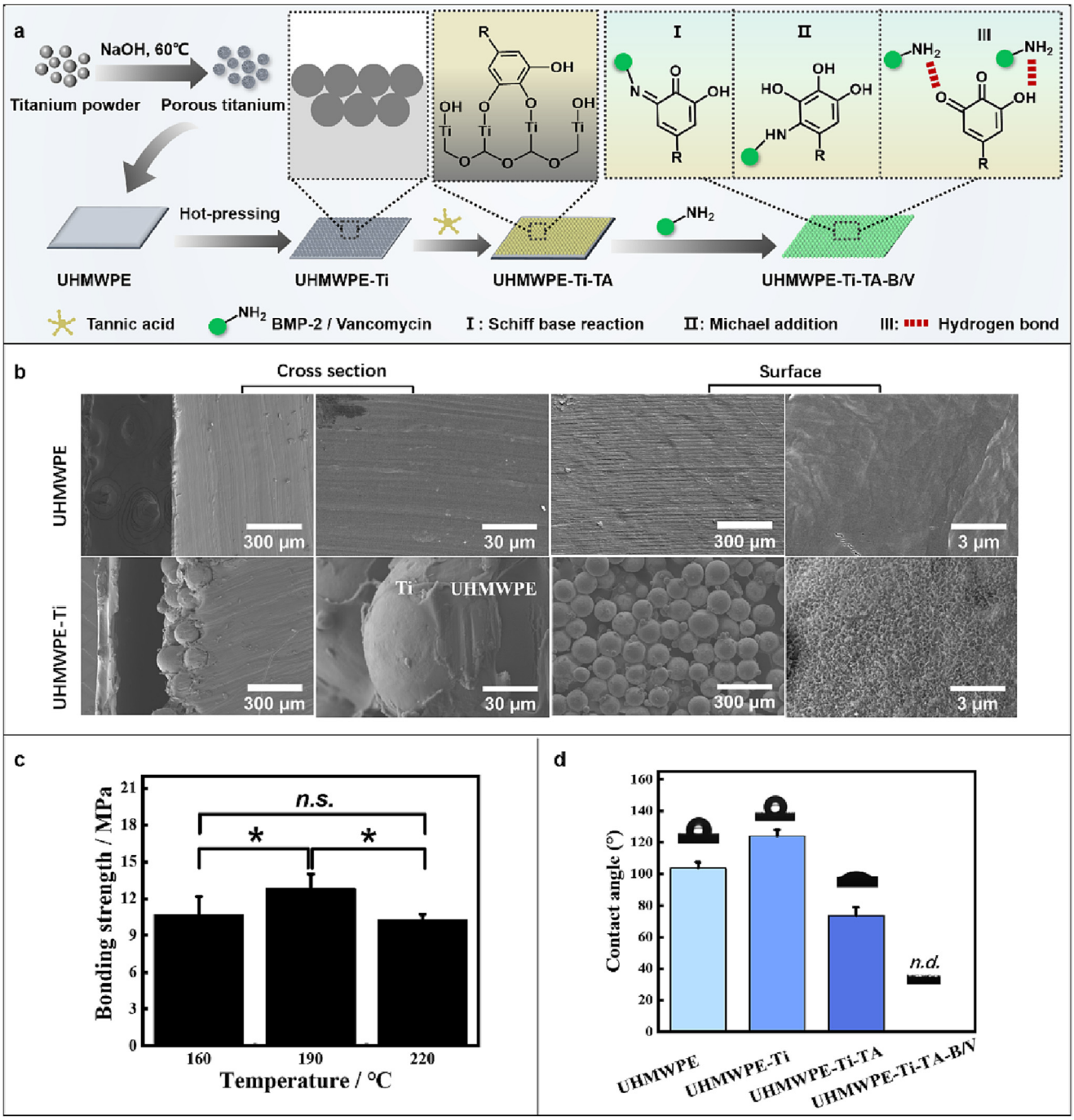

Fig. 2. (a) Schematic illustration of fabrication of porous titanium powders and biofunctional coatings on UHMWPE surface. (b) SEM images of cross-section and surface of UHMWPE and UHMWPE-Ti substrates. (c) Bonding strength of titanium layer to UHMWPE substrate prepared at different temperatures. *: $P < 0.05$, *n.s.*: no statistically significant difference between the groups. (d) Water contact angles of pristine UHMWPE, UHMWPE-Ti, UHMWPE-Ti-TA and UHWMPE-Ti-TA-B/V surfaces. *n.d.*: not detected.

UHMWPE-Ti and UHWMPE-Ti-TA disks. In contrast, an obvious zone of inhibition with a diameter of 1.41 cm was observed around the UHMWPE-Ti-TA-B/V disk. This result provides clear evidence that the UHMWPE-Ti-TA-B/V sample possesses good antibacterial property by diffusion of antibiotics from the substrate. Biofilm formation was further conducted to evaluate the antibacterial capability of the samples. As can be seen in Fig. 3b, the surface of pristine, and Ti- and TA-modified UHMWPE was heavily colonized by *S. aureus* after 24 h of culture. Almost no bacterial cell was observed on the surface of the titanium powder on the sample of UHWMPE-Ti-TA-B/V. It should be noted that bacterial cells might still exist in the pores of the UHMWPE/titanium hybrid layer. Therefore, the bacterial number on different samples was counted using the spread plate method. The viable bacteria number on the pristine UHMWPE surface was $\sim 1.11 \times 10^8$ CFU/cm^2, and it was 2.36×10^8 and 1.89×10^8 CFU/cm^2 on the UHMWPE-Ti and UHMWPE-Ti-TA surfaces, respectively (Fig. 3c), indicating the UHMWPE/titanium powder hybrid layer and TA treatment had little antibacterial effects. However, the viable bacteria reduced to $\sim 1.23 \times 10^4$ CFU/cm^2 on the surfaces of UHMWPE-Ti-TA-B/V substrate, which was about four orders of magnitude lower compared to that on pristine UHMWPE. The UHMWPE-Ti-TA-B/V sample

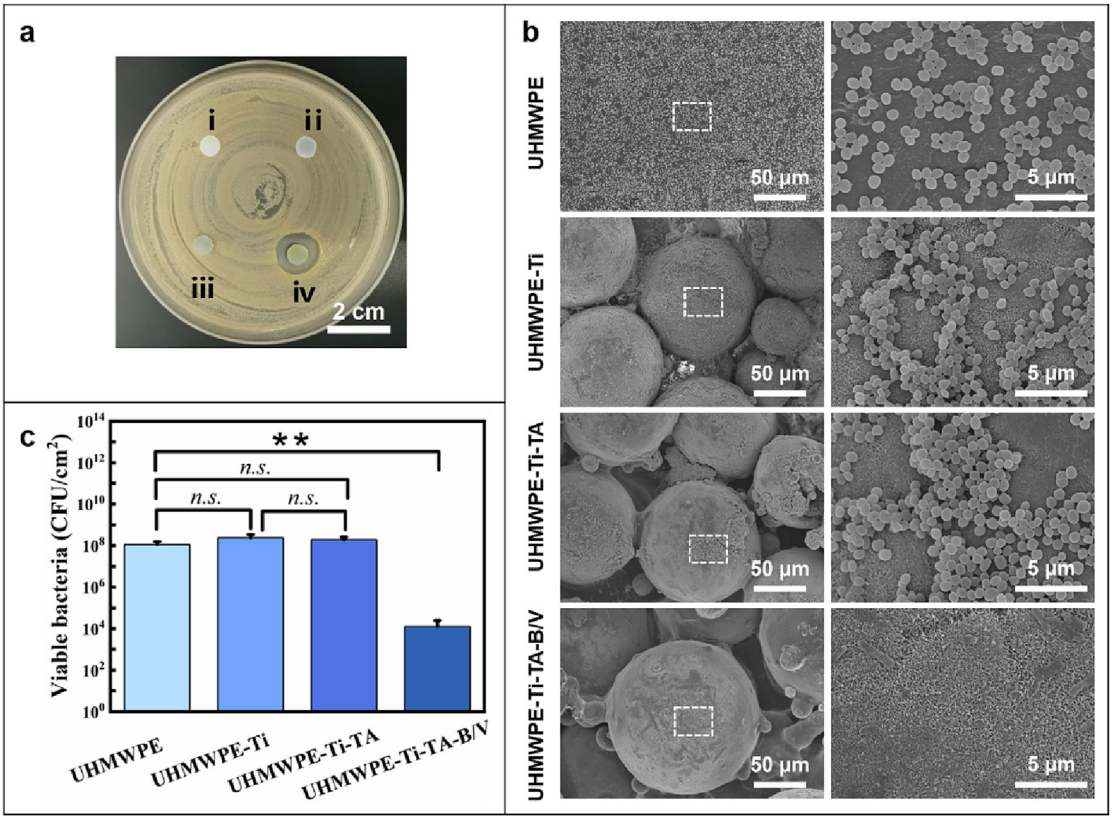

Fig. 3. *In vitro* antibacterial properties of different surface. (a) Inhibition zone of different UHMWPE plates, i: pristine UHMWPE, ii: UHMWPE-Ti, iii: UHMWPE-Ti -TA, iv: UHMWPE-Ti -TA-B/V. (b) SEM images of *S. aureus* bacterial biofilm on the pristine and modified UHMWPE surfaces after incubation for 24 h, the images in the right panel show the marked area in the left panel in a high magnitude. (c) Number of viable bacteria on pristine and modified UHMWPE surface after incubation for 24 h. **: $P < 0.01$, *n.s.*: no statistically significant difference between the groups.

exhibited strong inhibitory capability against *S. aureus*, probably due to the strong hydrophilicity of the surface prevented bacterial adhesion, and the antibacterial functions of vancomycin killed bacterial approaching the surface.

3.4. In vitro cytocompatibility

UHMWPE is a biologically inert material that lacks affinity with the surrounding bone tissue after implantation as an artificial joint, and loosening of the prosthesis is inevitable in long-term implantation [34]. Porous titanium is widely used in implants due to its good biocompatibility and superior mechanical property [17]. It has been demonstrated that titanium surface with appropriate porosity promoted bone integration and osteoinduction [36]. BMP-2 is an important extracellular signaling molecule that induces osteoblast differentiation and promotes osteogenesis [37]. Herein, the UHMWPE surface was modified with porous titanium by the hot-pressing method, and then grafted with BMP-2 to promote the adhesion, proliferation, and differentiation of osteoblast precursor cells. The surfaces of UHMWPE samples (pristine, UHMWPE-Ti, UHMWPE-TA, and UHMWPE-Ti-TA-B/V) were seeded with MC3T3-E1 cells and cultured for up to 7 days. SEM images of the cell adhesion and proliferation on the substrate surfaces were shown in Fig. 4a. As can be seen, there were few cells on the UHMWPE surface on Day 1, and no obvious cell proliferation was found on Day 4 and Day 7, indicating that the pristine UHMWPE surface was not conducive to cell adhesion and proliferation. Cell densities on the UHMWPE-Ti, and UHMWPE-Ti-TA-B/V surfaces were increased significantly on Day 4 and Day 7. A large number of cells with filamentous pseudopods adhered to the sample surfaces. This indicates that modification of the UHMWPE surface with biofunctional coatings could effectively improve the cell affinity of the samples, which is very important to improve the osseointegration ability of UHMWPE.

The cells on the UHMWPE samples were quantitatively evaluated using CCK-8 assay (Fig. 4b). After being cultured for 1 day, a significant increase of ~142% in the absorbance value in the UHMWPE-Ti group compared to the UHMWPE groups was observed, indicating a higher cell number on the UHMWPE-Ti surface. This is because that the porous titanium improved cell adhesion on the surface [38]. A slight decrease of cell density was found in the UHMWPE-Ti-TA group, probably due to that the TA layer on the surface had some cell inhibitory effect [39]. The cell density increased by ~132% on the surface that was coated with BMP-2 and vancomycin (UHMWPE-Ti-TA-B/V) compared to that on UHMWPE, indicating a beneficial effect of BMP-2 in promoting cell adhesion. After being cultured for 4 and 7 days, the cell density on the UHMWPE-Ti and UHMWPE-Ti-TA-B/V surfaces was significantly higher than that on the UHMWPE surface, showing a promoting effect of the surfaces to cell proliferation, which was consistent with the SEM results. For the UHMWPE-Ti-TA group, the cell density was comparable to the UHMWPE group on Day 7. To further investigate the cell functions and osteogenic differentiation level on Day 7, an ALP assay was performed (Fig. 4c). ALP is an enzymatic protein secreted by osteoblasts and is a specific marker of the maturation of osteoblasts [40]. As can be seen, compared to the UHMWPE group (ALP activity of 3.70×10^{-4} U/cm^2), the ALP activity of the UHMWPE-Ti groups was increased nearly two folds (7.16×10^{-4} U/cm^2), showing a great promoting effect of the porous titanium surface on osteoblast maturation. Although the cell

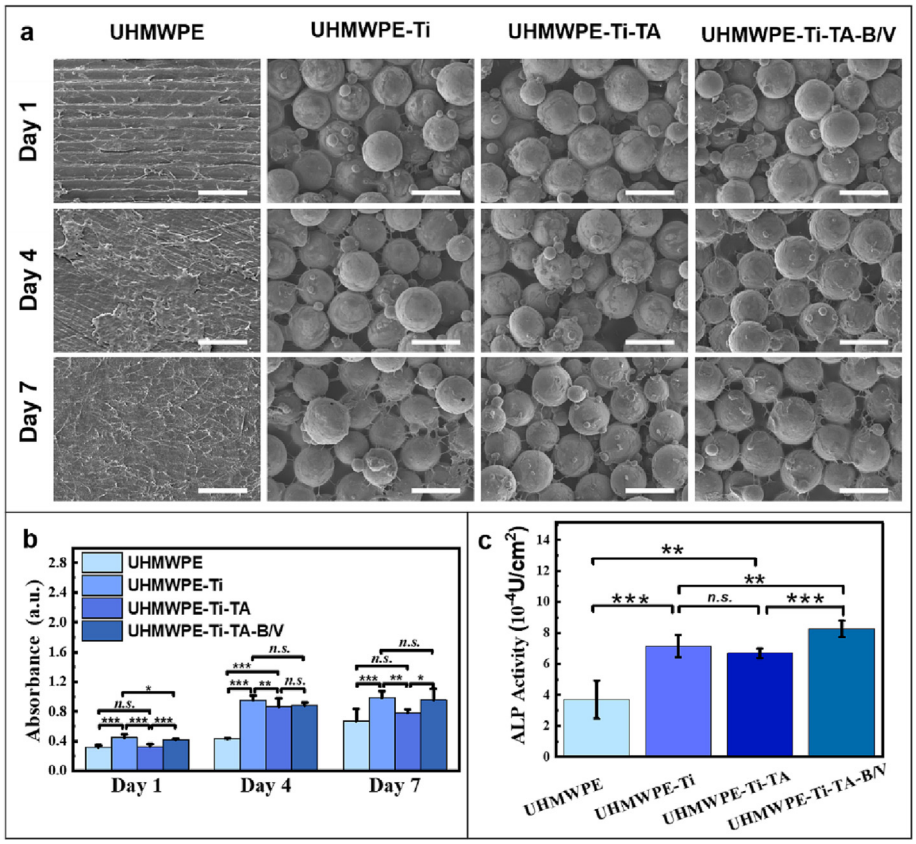

Fig. 4. *In vitro* cytocompatibility and cell proliferation. (a) SEM images of adhesion and proliferation of MC3T3-E1 cells on pristine and modified UHMWPE surfaces, scale bar: 150 μm. (b) Proliferation of MC3T3-E1 cells on pristine and modified UHMWPE surface as determined by CCK-8 assay. (c) ALP activity of MC3T3-E1 cells on pristine and modified UHMWPE surfaces after 7 days. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$, *n.s.*: no statistically significant difference between the groups.

number on the TA-coated surface (UHMWPE-Ti-TA) was slightly reduced (Fig. 4b), cell maturation on the surface was not inhibited (ALP activity of UHMWPE-Ti-TA group was 6.69 × 10^{-4} U/cm^2). The UHMWPE-Ti-TA-B/V group showed the highest ALP activity in all the groups (8.25 × 10^{-4} U/cm^2, ~223% of that in the UHMWPE group), confirming the significant beneficial effect of BMP-2 on the cell proliferation and maturation. In sum, the *in vitro* results confirmed that coating with porous titanium and grafting with BMP-2 growth factor greatly improved cell affinity of the UHMWPE surfaces, which was significantly important in artificial joint applications.

3.5. In vivo antibacterial study

Since the UHMWPE-Ti-TA-B/V sample exhibited excellent *in vitro* antibacterial and cell-promoting performance, UHMWPE-Ti-TA-B/V-coated disks were implanted subcutaneously in an infected rat model for 7 days to further evaluate the *in vivo* performance (Fig. 5a). The tissues surrounding the UHMWPE sample after 7 days were collected and homogenized, and the bacterial number in the tissue was quantified using the spread plate method (Fig. 5b). The bacterial number in the infected skin tissue from the rats with pristine UHMWPE sample was 3.12 × 10^4 CFU/g, while that from the rats with UHMWPE-Ti-TA-B/V sample was reduced by approximately two orders of magnitude, to 3.40 × 10^2 CFU/g. This suggests that the surface coating improved the antibacterial properties of UHMWPE, which is consistent with the *in vitro* antibacterial results, and leads to good biocompatibility with the surrounding tissues *in vivo*. The UHMWPE substrates with antimicrobial coating could be effective in avoiding the occurrence of bacterial infections in the implant site after artificial joint replacement surgery.

3.6. In vivo implantation study

Since the surface-modified UHMWPE showed excellent *in vitro* cellular activity and promoted osteoblast proliferation and differentiation, surface-coated UHMWPE acetabular cup was prepared and implanted in Labradors for 3 months to further investigate the histocompatibility and ability to promote bone tissue ingrowth. The outer surface of UHMWPE artificial acetabular cup was modified with porous titanium via the hot-pressing method using a customized mold, and then immobilized with BMP-2 and vancomycin. Fig. 6a showed the photographs of the UHMWPE acetabular cup before and after surface modification, and the shape of the cup remains unchanged after the modification procedures. Fig. 6b showed the micro-CT scanning of an evenly distributed titanium layer on the outer surface of the UHMWPE acetabular cup. The porous titanium-coated acetabular cup co-immobilized with BMP-2 and vancomycin was implanted into the acetabulum of the animal (Fig. 6c). All the animals implanted with the surface-modified acetabular cup were able to stand after 3 days, and walk after 14 days of the surgery. All three animals had good vital signs and no complications during the three months after the surgery. After three months, CT scanning of the implantation site revealed that the implanted cups in two of the animals were dislodged from the acetabulum. There are several possible reasons for the dislodgement of the acetabular cup, including unsuccessful surgery, an imperfect match between the animal and the acetabular cup, and excessive body movement by the animal. However, one

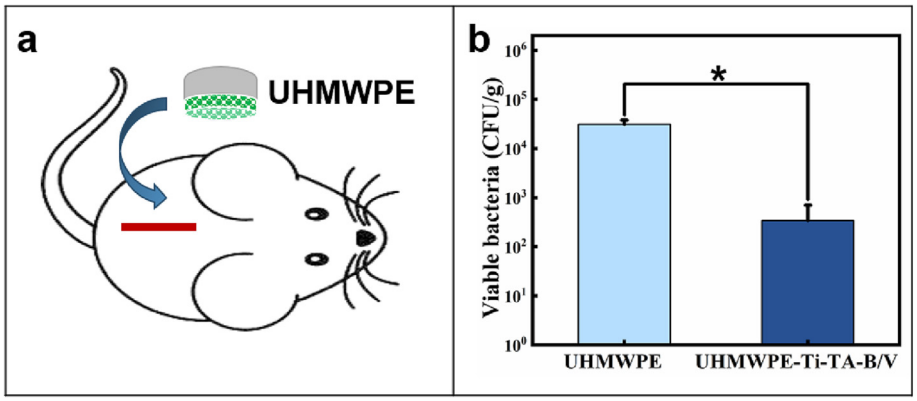

Fig. 5. (a) Schematic illustration of subcutaneous implantation of pristine and modified UHMWPE samples in rats. (b) Number of viable bacteria in the tissues around the implantation site with UHMWPE and UHMWPE-Ti-TA-B/V after 7 days. *: $P < 0.05$.

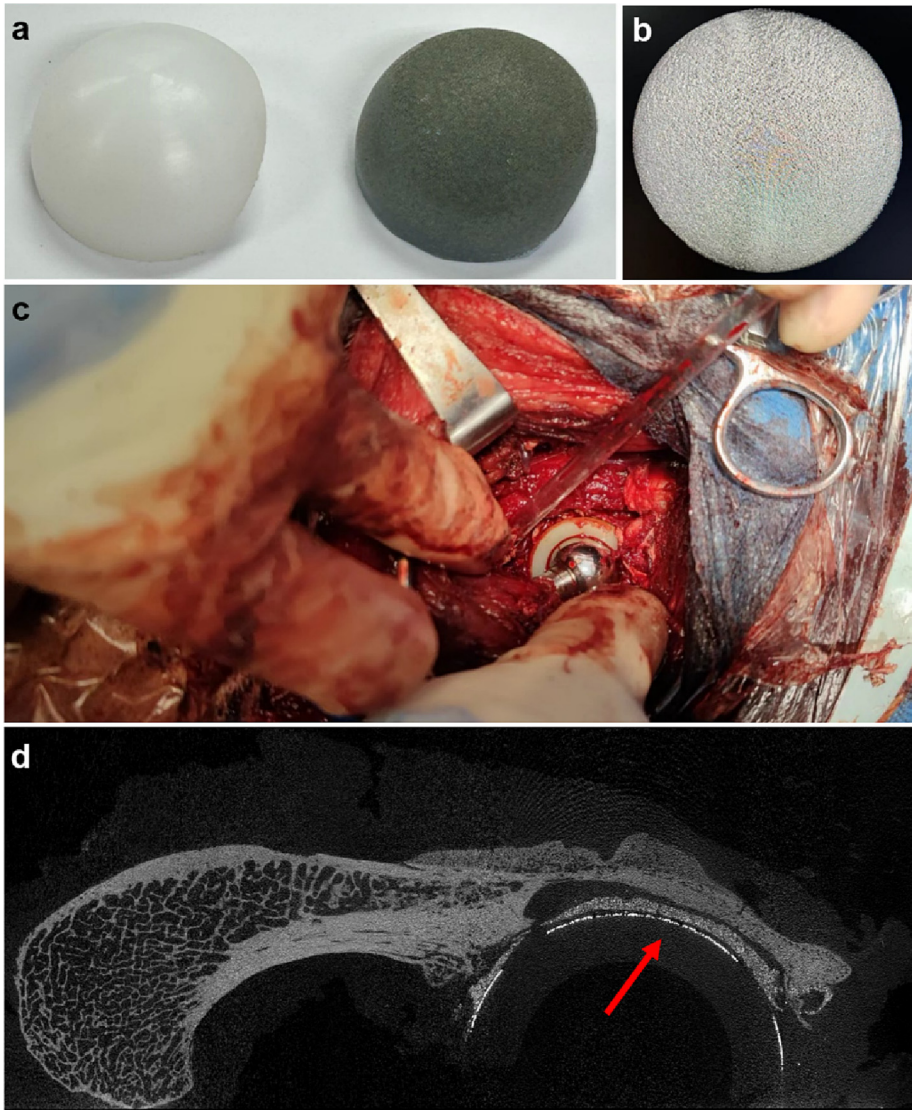

Fig. 6. (a) Photographs of UHMWPE acetabular cup before (left) and after (right) surface modification. (b) Morphological structure of porous titanium layer on acetabular cup characterized by micro-CT scanning. (c) Implantation of surface-modified acetabular cup in the animal, and (d) micro-CT image of acetabular cup implant site after three months, the red arrow marks the interface between the convex surface of the cup and the acetabulum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acetabular cup in the animal remained stable in the acetabulum after three months. Further micro-CT scanning revealed an initiation of bone ingrowth and integration of the acetabular cup surface with the surrounding bone tissues (Fig. 6d), which had a bone volume fraction (BV/TV) of 1.097%, specific bone surface (BS/TV) of 0.658 mm^{-1}, a trabecular thickness (Tb.Th) of 0.143 mm, a trabecular number (Tb.N) of 0.077 trabeculae/mm, and a trabecular separation (Tb.Sp) of 4.001 mm. This indicates that the surface-modified artificial acetabular cup had good biocompatibility and biofunctionality with the surrounding tissues, and may be used as a bioactive implant *in vivo*. It should be noted that the number of animals and implant duration in this study were limited, and the surgical technique also needs to be improved. In the future, a larger number of animals with more sophisticated surgical procedures and longer implant durations should be involved to investigate the surface-biofunctionalized acetabular cup in a more comprehensive manner.

4. Conclusions

In this study, we developed a facile and convenient strategy to modify UHMWPE surface with coatings to endow it with osteogenic and antibacterial properties. A porous titanium layer was formed on UHMWPE surface by the hot-pressing process, and the bonding strength between the titanium layer and the substrate was up to 12.86 MPa, which was higher than the reported tensile stresses within the acetabulum. The UHMWPE with a porous titanium layer was treated with TA and then co-immobilized with vancomycin and BMP-2. The porous titanium-coated and antibiotic- and growth factor-grafted UHMWPE surface showed significant capability to promote the adhesion, proliferation, and differentiation of osteoblast precursor cells, which may be related to the porous structure of the titanium layer, as well as the presence of BMP-2. Due to the antibacterial function of vancomycin and the super hydrophilic property, the modified UHMWPE exhibited excellent antibacterial properties. There were no adverse reactions in the animals after implantation of the artificial acetabular cup over three months. Although two acetabular cups dislodged in the animals, one of the cups remained stable in the acetabulum. The surface of the cup was integrated with the bone tissues in the acetabulum, indicating good bioactivity of the implant. In summary, the coating of porous titanium layer co-immobilized with BMP-2 and vancomycin on UHMWPE has great potential as an artificial joint prosthesis material in clinic practice.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matdes.2023.112131.

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