3D Printed Ti–6Al–4V Implant with a Micro/Nanostructured Surface and Its Cellular Responses

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ABSTRACT: Three-dimensional (3D) printing technology has been proved to be a powerful tool for the free-form fabrication of titanium (Ti) implants. However, the surface quality of 3D printed Ti implants is not suitable for clinical application directly. Therefore, surface modification of 3D printed Ti implants is required in order to achieve good biocompatibility and osseointegration. In this study, a novel surface modification method of 3D printed Ti–6Al–4V implants has been proposed, which combined acid etching with hydrothermal treatment to construct micro/nanostructures. Polished TC4 sheets (P), electron beam melting Ti sheets (AE), and micro/nanostructured Ti sheets (AMH) were used in this study to evaluate the effects of different surface morphologies on cellular responses. The surface morphology and 3D topography after treatment were detected via scanning electron microscopy and laser scanning microscopy. The results illustrated that a hierarchical structure comprising micro-valleys and nanowires with a surface roughness of 14.388 μm was successfully constructed. Compared with group P samples, the hydrophilicity of group AMH samples significantly increased with a reduced water contact angle from 54.9° to 4.5°. Cell culture experiments indicated that the micro/nanostructures on the material surface could enhance the cell adhesion and proliferation of MC3T3s. The microstructure could enhance bone-to-implant contact, and the nanostructure could directly interact with some cell membrane receptors. Overall, this study proposes a new strategy to construct micro/nanostructures on the surface of 3D printed Ti–6Al–4V implants and may further serve as a potential modification method for better osteogenesis ability.

1. INTRODUCTION

With the aging of the population and the increase of traffic accidents, orthopedic operations are gradually increasing. Although natural bone tissue is capable of self-healing, it is necessary for patients with critical bone defects to use artificial implants to assist in tissue rebuilding. How to quickly design and prepare an implant of the shape required by the patient has become a major clinical problem. The layer-by-layer manufacturing method of 3D printing technology, which can fabricate arbitrary 3D shapes, provides a way to solve this problem. In general, 3D printing technology is one of the most promising manufacturing methods for bone defects due to its advantages of free-form fabrication, material saving, and shortened production cycle.

Numerous bone repair materials are being studied to promote osseointegration between implants and the bone repaired. Ti and its alloys are widely used in dentistry and orthopedics implants due to their excellent chemical stability, outstanding biocompatibility, and mechanical properties. However, Ti as a bioinert material leads to poor osseointegration with nature bone tissue. In metal 3D printing process, energy is used to melt metal powders following a predetermined scanning path and form the final shape layer by layer. Unfortunately, a large amount of residual powders melted imperfectly will attach to the substrate after printing. Residual powders can detach from the implant surface into the humoral system causing osteolysis. Therefore, it is necessary to post-process the 3D printed Ti surface in order to improve its biological behavior.

Nature bone is a precise multistage complex which is composed of macro-, micro-, and nano-scale structures. From a biomimetic viewpoint, the surface property of implants plays an essential role in promoting its biocompatibility. In the past few years, many researchers have focused on surface modification via changing the physical and/or chemical characteristics. Generally, there are two ways to improve the surface properties of Ti implants. One is microstructure modification; typical techniques are sandblasting and acid...
etching, micro-machining, micro-arc oxidation, and plasma electrolytic oxidation. The other is nanostructure modification using hydrothermal method and anodic oxidation. Implants with microstructures and/or nanostructures show great potential in enhancing bioactivity and osseointegration. The difference is that microstructures contribute to enhance the binding force and contact area between the implant and bone to reduce the loosening of the implant. Nanostructures could raise the adsorption of proteins and promote the differentiation of mesenchymal stem cells into osteoblasts. Zhao et al. and Wang et al. have constructed micro/nanostructures by producing titania nanotubes on micro-topographies treated with micro-milling and anodic oxidation, which promotes several cellular behaviors such as initial cell adhesion, alkaline phosphatase activity, and proliferation. What is more, Wang et al. have studied the role of the Wnt/β-catenin pathway on micro/nanostructured surfaces. Micro/nanostructures can enhance the expression of Wnt protein and inhibit the expression of SFRP1, SFRP2, DKK1, and DDK2 to promote cell adhesion and proliferation.

Recently, 3D printing of implants has drawn remarkable attention because its unique features match clinical requirements. However, most of the previous studies were focused on printing performances and internal microstructures to achieve better mechanical properties and avoid serious stress-shielding effect. Further investigations about the construction of micro/nanostructures and their effects on cellular behaviors are still needed to improve the biocompatibility of 3D printed implants. Therefore, in this study, the micro/nanostructure was fabricated on the surface of 3D printed Ti–6Al–4V by...
acid etching and hydrothermal treatment. The influence of surface micro/nanostructures on cellular behaviors was also investigated.

2. RESULTS

2.1. Surface Characterization. The construction steps of the micro/nanostructures on the 3D printed Ti−6Al−4V surface are presented in Figure 1. The surface morphologies of different samples are shown in Figure 2. It can be seen that group P exhibited a flat surface after polishing (Figure 2a−c). In contrast, group AE at low magnification (Figure 2d) showed a rough surface with transverse valley-shaped structures. At high magnification, micro-grooves distribute on the surface (Figure 2e,f) due to acid etching; widths of these microstructures mostly range from 0.3 to 0.9 μm. After hydrothermal treatment, micro-valley structures caused by electron beam scanning still remained on the surface of the group AMH under low magnification (Figure 2g). At high magnification (Figure 2h,i), cluster-shaped structures composed of nanowires with a diameter of 30 nm were observed. The experiment results show that the micro/nanostructure was successfully fabricated after acid etching and hydrothermal treatment on the 3D printed Ti surface.

The 3D topographies and surface roughness are shown in Figures 3 and 4. As expected, the surface of group P sample was flat with the Sa value of 0.652 μm, which was in accordance with the result of SEM. After acid corrosion, the measured Sa value of group AE was 13.702 μm. The increase of surface roughness is due to the existence of micro-valleys (Figure 3b). Compared with AE samples, the surface roughness of group AMH was slightly increased (Sa = 14.388 μm) due to hydrothermal treatment. All in all, 3D printing technology provides a surface which is more similar to nature bone than the smooth polished Ti.

The crystalline phases of group P, group AE, and group AMH substrates are displayed in Figure 5 to determine the phase transformation. It is clearly observed that there is no significant difference between group P and group AE. However, an additional diffraction peak was detected on the AMH surface, which was anatase.

Surface wettability played a pivotal role in mediating cell response and protein adsorption. The contact angles of group P, group AE, and group AMH are shown in Figure 6. Apparently, the contact angle of group P was 54.9°, implying that the polished Ti surface was hydrophilic. Group AE showed a hydrophobic surface with a contact angle of 101.9°. After hydrothermal treatment, the contact angle of group AMH significantly decreased to around 4.5°. The reason for this is the formation of nanowires on the surface.

2.2. Cell Proliferation and Cell Morphology. Cell proliferation on different Ti sample surfaces has been assessed and shown in Figure 7. There was no significant difference among the three kinds of Ti samples at day 1. At day 4, the cell viability of group P surface was statistically lower than that of group AMH (*p < 0.05). After culturing for 7 days, the cell viability of group AE and AMH was statistically higher than that of group P. The microstructure led to better cell viability in group AE than group P. The viability of cell proliferation to group AMH substrates was slightly higher than that of group AE. These results indicate that the micro/nanostructured surface has a positive effect on cell proliferation due to the synergistic effect of micro-valleys and nanowires.

The morphology of cell adhesion on different Ti sample surfaces was evaluated. The result is shown in Figure 8. MC3T3s on the surface of group P exhibited oval-like morphology without filopodia. In contrast, MC3T3s of group
AE and group AMH displayed noticeable filopodia extensions. Besides, the cells of group AMH displayed a larger spreading area than those on the AE. It is indicated that the micro/nanostructured surface is more propitious to cell spreading due to the synergistic effect of the microstructure and nanostructure.

3. DISCUSSION

The surface of the implant with micro/nanostructure plays a pivotal role in the osseointegration and could decline implant looseness and improve the success rate of implant operation. The regular micron surface modification method is sandblasting and acid-etching, which leads to a waste of energy. Traces of electron beam scanning can obviously raise the roughness of the implant without any micro-scale postprocessing. Therefore, this study is dedicated to achieving a micro/nanostructure on the surface of 3D printed Ti−6Al−4V implants to improve the responses of osteoblasts.

Previous studies mainly focused on the design and topological optimization of the lattice structure to achieve a better elastic modulus or permeability and thus better osseointegration. However, in this study, we focused on modifying the surface topography of 3D printed implants to improve osseointegration. Therefore, a novel surface modification method was proposed to construct micro/nanostructures on the surface of 3D printed implants. First, a 3D printed sample was treated by acid etching to remove residual powders attached on the surface. Then, the nanowire structure was superimposed on the surface via a hydrothermal reaction of hydrogen peroxide. After annealing treatment, anatase TiO2 nanowires were observed, which have proved to be greatly bioactive. Roy et al. found that anatase enhanced the corrosion resistance, which could slow down the corrosion of the implant in the human body, thus extending the service life of the implant. In terms of wettability, hydrophilia played a pivotal role in promoting osseointegration as reported by Liu et al. The surface of group AE was hydrophobic with a contact angle of 101.9°. The water contact angle of group AMH was 4.5° due to the effect of nanowires, showing excellent hydrophilia.

The effects of the micro/nanostructured surface on the cell proliferation and adhesion were evaluated in vitro. The proliferation on the group AE and group AMH surface were better than those on the group P surface due to the effect of microstructure and nanostructure morphologies. In terms of cell adhesion, there were more filopodia on the surface of group AMH. As a result, micro/nanostructures have a positive effect on promoting the proliferation and adhesion of cells.

4. CONCLUSIONS

In this study, the micro/nanostructure composed of valleys and nanowires was constructed on 3D printed Ti−6Al−4V surface by the combination of acid etching and hydrothermal method. Compared with group P, the surface roughness and hydrophilicity of group AE and group AMH were greatly improved. More importantly, the micro/nanostructured
sample was conducive to enhance the proliferation and adhesion of MC3T3-E1. Overall, this study provides an effective method for improving osteoblast responses by modifying the surface of 3D printed Ti implants, which has the potential to advance the application of 3D printing technology in clinics in the future.

5. EXPERIMENTAL SECTION

5.1. Sample Preparation. A 3D printing machine (Q10 plus, Arcam, Sweden) was used to construct the Ti–6Al–4V sheets with dimensions of 10 × 10 × 1 mm³ by electron beam melting technique. The diameter of Ti–6Al–4V powders ranged from 45 to 106 μm. The pre-heating temperature of the building chamber was 750 °C. The scanning velocity was 800 mm s⁻¹, and the beam diameter was 100 μm. All samples were immersed in a mixed solution of HF and HNO₃ (v/v/v = 1:3:6, 40% HF, 65% HNO₃, and H₂O₂) for 2 min.

Half of group AE samples was soaked in a mixed solution of H₂O₂, HNO₃, and melamine (H₂O₂ 50 mL, HNO₃ 1 mL, and melamine 100 mg) in a teflon-sealed autoclave for 24 h at 80 °C to obtain AMH substrate. After that, the samples of group AMH were annealed at 450 °C for 2 h. In summary, the fabrication process of micro/nanostructure is shown in Figure 1. Moreover, commercially available Ti–6Al–4V sheets (TC4, Baoji Titanium Industry, Baoji, China) were cut into rectangular samples as the control group, the size of which was 10 × 10 × 1 mm³. Rectangular samples were polished with abrasive paper from 400# to 2000#.

5.2. Surface Characterization. The surface morphology of samples was characterized by SEM (XSM-7610F, Japan). The 3D topographies and surface roughness of different samples were measured by a 3D laser scanning microscope (LSM, VKX200K, Japan). A contact angle goniometer (LSM, VKX200K, Japan) was used to evaluate the surface wettability with 2 μL deionized water droplets. The crystalline phases were determined by XRD (D8 Advance, Germany).

5.3. Cell Proliferation. The mouse osteoblast cell line MC3T3-E1 was cultured in α-minimum essential medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C under 5% CO₂ atmosphere. After incubation for 1, 4, and 7 days in 24-well plates at the density of 1 × 10⁴/mL, each sample was added into 400 μL of fresh medium with 100 μL of MTT and incubated for 4 h. Then, 400 μL of dimethyl sulfoxide was added into each well. After 10 min, 200 μL of mixed solution was taken out into a new 96-well plate to measure the absorbance value (optical density) by a spectrophotometric microplate reader (Thermo Labsystems, America) at 490 nm.

5.4. Cell Morphology. MC3T3-E1 cells were seeded on different Ti samples placed in new 24-well plates at the density of 5 × 10⁴ for 2 days. The cell-adhered specimens were fixed with 4% paraformaldehyde for 25 min at 4 °C. Next, the samples were rinsed with phosphate-buffered saline and permeabilized with 0.1% Triton-X100 for 10 min. Afterward, the nuclei were stained with 400 μL of tetramethyl rhodamine-labeled rhodamine-phalloidin (5 U mL⁻¹) in darkness for 90 min at room temperature and cytoskeletons were stained with Hoechst 33258 for 15 min. The stained samples were observed with a confocal laser scanning microscope (LSM 780, Carl Zeiss, Germany).

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Notes

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