The Effect of TiAl6V4 Particles on Tissue in Rats

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Abstract. Electron beam melting (EBM) is three-dimensional (3D) printing technologies that can manufacture multi-functional porous scaffolds with exact structures for the application of surgical operations. In the past decade, thousands of acetabular implants manufactured by EBM or SLM have been designed into acetabular cups with certain porosity for surgical operations. Particles of 3D printed porous Ti6Al4V implants will adhere between sintered interface and non-sintered interface of porous sample during 3D printing. However, the internal excess particles of complex structural parts are difficult to remove. During long-term cyclic loading, stress strain can cause residual Ti6Al4V particles to fall off. These detached Ti6Al4V particles are scattered around the implant and are contact with osteoblasts. In our study, we detected the influence of the differentiation concentration of Ti6Al4V particles on bone of rats. The influence of the differentiation concentration of Ti6Al4V particles on bone was evaluated by distal femoral defects in rats. Micro-CT and biochemical analysis were used to evaluate all of the rats after 12 weeks. Outcomes demonstrated that low concentration Ti6Al4V particles may improve the osteogenesis of SD rat through micro-CT. Serum markers implied that the differentiation concentration of Ti6Al4V particles didn’t influence on hepatic and renal functions. In conclusion, Low-dose residual particle does not induce a decrease in bone mineral density (BMD) of rats.

1. Introduction

Ti6Al4V has good corrosion resistance and biocompatibility after being implanted in the body, so it is widely used as a material for orthopedic equipment[1]. An increasingly important role has been played by Ti6Al4V in biomedical applications recently[2]. EBM or selective laser melting (SLM) have permitted the use of computer-aided design (CAD) software combined with patient microcomputer tomography to manufacture targeted Patient's metal or alloy implant [3,4,5]. Complex three dimensional
(3D) metal parts and fabricate porous Ti6Al4V implants can be fabricated by EBM, which offer a safe manufacture process in improved control for internal structures and external shapes[6,7]. Porous Ti6Al4V implants have good mechanical properties and biocompatibility[8,9,10]. However, Ti6Al4V particles will adhere between sintered interface and non-sintered interface of 3D printed porous sample. Internal residual particles are difficult to remove. During long-term cyclic loading, stress strain can cause residual Ti6Al4V particles in the pores of the implant to fall off. The detached residual Ti6Al4V particles can directly touch the tissues and cells surrounding the implantable medical materiel and may affect dynamic balance of bone formation and bone resorption. Previous studies have proved pathological response could be induced by wearing nanoparticles[11], including changes of aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT), inflammatory cell infiltration and necrosis[3], like alternative modus of cell apoptosis, death has also been informed[11,4]. However, the interaction between osteoblasts and residual Ti6Al4V particles remains largely unknown.

Clinically, long-term stress loading causes a small amount of detachment of residual Ti6Al4V particles in the implant. These detached Ti6Al4V particles are scattered around the implant and are in extensive contact with osteoblasts. Our research purposed to investigate the influence of differentiation concentration of Ti6Al4V particles on bone of rats. In this study, the rats were used to investigate the bone growth with Ti6Al4V particles by the micro-CT. Our research also investigated the influence of Ti6Al4V particles on other tissues of rats to determine the optimal concentration of Ti6Al4V particles about biological compatibility.

2. Material and Methods

2.1. Manufacture and Description of Ti6Al4V Particles
Ti6Al4V particles were obtained from porous implant fabricated through EBM. Ultrasonic cleaning and sterilized were used to dispose all the particles. Then Ti6Al4V particles were suspended in minimal medium (Hyclone, USA). The Ti6Al4V particles diameter was between 30-70 microns, and the particle was homogeneous without cracks (figure1.(a)-(b)). The scanning electron microscope (SEM, Helions, FEI, USA) was used to scanned the surface characterization of Ti6Al4V particles in porous samples.

![Figure 1. SEM morphologies of particles on porous samples](image)

2.2. Animals and Surgical Procedures
We purchased 32 female 8 weeks old Sprague Dawley (SD) rats (weight 250–270 g) from experimental animal center of Beijing University. The SD rats were given the same housing/feeding conditions for 12 weeks in the animal center of Beihang University, China. Relevant laws and regulations (including Administration of Affairs Concerning Experimental Animals promulgated and the Guiding Principles for the Care and Use of Animals approved) were carried out to care all SD rats. The Animal Care Committee in Beihang University also approved all of the protocols. 1% pentobarbital sodium (6 ml/kg, i.p.) was used to anesthetized SD rats for surgery. We drilled the cylindrical defects in the right distal femurs, and placed the Ti6Al4V particles into cylindrical defects. We randomly divided thirty-two SD
rats into four groups (n=8, each group): 1mg/100g of the Ti6Al4V particles were in the defects low group, 5mg/100g of the Ti6Al4V particles were in the defects middle group, 25mg/100g of the Ti6Al4V particles were in the defects high group and control (CON) group. Overdose of an anesthetic was used to inject to sacrifice SD rats after 12 weeks.

2.3. BMD detected through Micro-CT
On day 85, 1% pentobarbital sodium (6 ml/kg, i.p.) was used to anaesthetize the rats for micro-CT scanning (SkyScan1076, Belgium) in vivo. The micro-CT was used to scan distal femurs of rats[12]. Scan parameters was 143 μA current, 70 kV X-ray voltage and 18 μm pixel. The same parameters were used to reconstruct all scan data. The same investigator delineated the region of interest. Then we calculated every cortical BMD and the trabecular BMD in the right distal femurs.

2.4. Statistical Analysis
Means ± standard deviation (SD) presented all values. One way ANOVA analysis of SPSS 15.0 was performed. P<0.05 was the level of statistical significance.

3. Results
3.1. BMD by Micro-CT
On day 85, cortical BMD of the Low group significantly raised contrasted with that of CON. There were no significant among CON group, Middle and High group. Trabecular BMD of the Low, Middle and High group were lower than that of CON, but no significant differences were note (figure 3). *indicates significant difference versus CON.

**Figure 2.** Particles implantation in SD rat
3.2. Serum Markers

Figure 4 The changes of the biochemical parameters of the serum in the rats were showed induced by implants in Low, Middle and High groups. There was no significant variation in indicators (BUN and CRE) of the kidney function in the four groups. Similarly, there was no significant variation in indicators (AST and γ-GT) of their liver function.

4. Discussion

During the wear of artificial implants, a large number of wear particles can be released. These particles can induce osteoclast formation and osteolysis around the prosthesis. Moreover, Wear particles penetrate into the tissue surrounding the implant, elevating inflammation and resorption of bone[13]. Therefore, periprosthetic osteolysis induced by wear particles is one of the main reasons for implants failure[14]. Osteoblasts are the main cells that maintain bone metabolism. Porous Ti6Al4V could
promote osteoblast differentiation[15,16]. Porous titanium implants with good biocompatibility, reliable mechanical properties and corrosion resistance, therefore has been widely used in orthopaedic or not in the healing of bone defect of orthopaedic implants. [17,18]. However, residual particle inside a porous implant with a complex structure cannot be completely removed, and will continue to fall off over time. These exfoliated titanium alloy particles are dispersed around the implant and are in extensive contact with osteoblasts.

Micro-CT indicated that there was an increase in cortical BMD in the Low group compared to that of CON at 12 weeks after implantation. The results of our study showed that the low concentration Ti6Al4V particles may substantially improve the osteogenesis. Furthermore, the normal levels of serum showed that Ti6Al4V particles didn’t inducing infect and necrosis in the three implant groups. The results of our study suggested that the functions of liver and kidney didn’t be affected by the implants. This study suggested that small amount of residual powder may be beneficial for bone growth.

5. Appreciation
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6. About Interest
All authors declare no conflicts of interest.

7. References
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