3D printed Ti6Al4V bone scaffolds with different pore structure effects on bone ingrowth

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Abstract

The pore size, pore structure and porosity of porous scaffold play a vital role in bone regeneration, but its optimal shape is still unclear. In this study four kinds of porous titanium alloy scaffolds with similar porosity (65%) and pore size (650µm) and different structures were prepared by selective laser melting. Four scaffolds were implanted into the distal femur of rabbits to evaluate their bone tissue growth in vivo. Micro-CT and hard tissue sections were performed 6 and 12 weeks after the operation to reveal the bone growth of the porous scaffold. The results show that diamond lattice unit (DIA) bone growth is the best in four topological scaffolds. Through computational fluid dynamics (CFD) analysis, the permeability, velocity and flow trajectory inside the scaffold structure were calculated. The internal fluid velocity difference of the DIA structure is the smallest, and the trajectory of fluid flow inside the scaffold is the longest. It is beneficial to the growth of blood vessels and the transport of nutrients, and can promote bone formation. In this study, the mechanism of bone growth in different structures was revealed by the method of in vivo experiment combined with CFD, providing a new theoretical basis for the design of bone scaffolds in the future.

Keywords: Selective laser melting; Bone scaffold; Pore geometry; Computational fluid dynamics; bone ingrowth

1. Introduction

In recent years, due to its good biocompatibility and corrosion resistance, titanium and its alloys have been widely used in clinics\cite{1}. The application of titanium bone scaffolds in bone defect repair has attracted wide attention and is considered as a feasible method to repair and replace bone defect beyond critical defect value. Titanium alloy as a low modulus metal (young’s modulus range from 7 to 30 GPa) \cite{2}. Although the elastic modulus of titanium is still larger than that of human bone, compared with tantalum, stainless steel and other metal materials, the stress masking effect caused by the mismatch of elastic modulus is much smaller. The matching degree of titanium implants with host bone is also higher, and the incidence of bone resorption and implant loosening is also reduced \cite{3}. Porous structure is considered to be an effective method to eliminate elastic modulus mismatch \cite{4-6}. Porous titanium scaffolds with low elastic modulus can further reduce the stress masking effect, fit well with host bone, reduce bone absorption, and
promote rapid bone formation and integration at the bone-implant interface [7]. Because it is difficult to accurately control the pore size, porosity, pore shape, and pore connectivity with traditional manufacturing techniques, the microstructure of the constructed porous structure is uncontrollable [8]. In recent years, with the rapid development of 3D printing technology, such as selective laser melting (SLM), selective laser sintering, electron beam melting and other technologies have made it possible to prepare porous structures with controllable microstructures [9-12]. Based on the computer-aided design (CAD) modeling method, the CAD technology is used to create a porous microstructure model with controllable porosity and connectivity. The advantage is that the model is relatively simple to build, which is convenient for mechanical analysis, and 3D printing technology can be used to quickly materialize the model. Therefore, the research on 3D printed porous bone scaffolds has been high in recent years.

Existing research mainly focuses on the design of porous scaffolds with mechanical property that match the host bone tissue. For different porous structures, the researchers studied the effect of pore size or porosity on bone ingrowth. After the mechanical property are matched with the host bone tissue, the subtle changes in the pore shape have a great influence on the adhesion and proliferation of bone tissue cells. The pore shape of the scaffold plays a decisive role in cell growth. Arun et al. [13] conducted an overall study on the stiffness, strength, permeability and stress concentration of the six scaffold structures with a porosity of 68.46 - 90.98%. The results show that the pore shape affects the permeability, stiffness, strength and strength of the Ti6Al4V bone scaffold Stress concentration factor. The research of Bidan et al. [14] shows that the optimization of the shape of the pore size can increase the growth rate of bone tissue to the porous scaffold, and the cells grow faster in the square pores. Bael et al. [15] studied the local curvature and pore shape, and the results showed that obtuse Angle was more likely to cause cell blockage than acute Angle. The results of Urda et al. [16] indicate that the straight edges and convexities in the pore structure are the most unfavorable for cell growth. However, these studies on porous scaffold structures are limited to cell experiments, and the growth of cells in vivo is very different from that of cell culture in vitro. Therefore, it is of certain guiding significance for the design of bone tissue scaffolds in the future to analyze the osteogenic performance of porous bone scaffold shape in vivo.

Pore size and porosity also have a great influence on the bone ingrowth. Weihu Yang[11] et al. prepared a porous titanium scaffold with a pore diameter of about 650 µm by selective laser melting technology, which had better bone ingrowth and better bone integration at the bone-scaffold interface than those with a pore size of 500 and 900 µm. Xijing He[5] et al. prepared a porous titanium scaffold with a pore diameter of 650 µm by selective laser melting technology, which had a better bone growth effect than those with a pore size of 400, 500, and 1100 µm. Taniguchi et al. [17] showed that SLM porous titanium scaffolds with a porosity of about 65% and a pore diameter of about 600 µm could achieve better stability and bone growth compared with SLM porous Ti6Al4V implants with a pore diameter of 300 µm and 900 µm. In fact, higher porosity and pore size is more conducive to bone ingrowth, although this will reduce the strength of the scaffold [18,19]. Therefore, when designing scaffolds, we should choose scaffolds with high porosity and pore size to improve bone growth while ensuring scaffold strength.

In this paper, four common porous structures (DIA, TC, CIR, CU) with a pore size of 650 µm and a porosity of 65% were selected and designed by CAD software. After SLM was used to manufacture the scaffolds, the mechanical experiments showed that the scaffolds had sufficient
strength to meet the needs of in vivo experiments. After implanting it into the distal femur of the rabbit, Micro-CT and hard tissue sections were performed at 6 and 12 weeks, respectively to evaluate the bone ingrowth in vivo. Using computer fluid dynamics (CFD) method to study the relationship between pore structure parameters and hydrodynamic strutures, from the perspective of fluid mechanics to reveal the impact of pore structure on bone formation performance. scaffold Thus, the optimized porous structure can be determined to provide theoretical scaffold for further research on 3D printed porous bone scaffolds suitable for human body.

2. Materials and methods

2.1 Design and manufacturing of scaffolds

Unigraphics NX (Siemens PLM Software, Germany) was used to design four common porous scaffolds (DIA,TC,CIR, CU) with a porosity of 65% and a pore size of about 650 µm. The porosity of the scaffold is the percentage of the structure in a complete solid: $V_p/V_s$

Where, $V_p$ is the volume of structural unit, and $V_s$ is the volume of complete solid.

In order to obtain the structure with the same porosity and pore size, we conducted parametric design for the design parameters of all structures, as shown in Fig. 1. The pore size of the structure is ‘t’, the unit length is ‘a’, and the strut diameter is ‘D’. The pore size t of the structure is determined by means of the maximum tangential circle. All structures are regular geometric structures, so a mathematical relationship between design parameters and porosity and pore size can be established. Since the CIR model is not constructed using struts, the pore size t is used to represent its volume.

$$V_{DIA} = 4\pi D^2 - \frac{585}{1024} D^3 \tan\left(\frac{1}{2} \arccos\left(\frac{1}{3}\right)\right) - \frac{15\sqrt{6}}{128} \pi D^3 + \frac{135}{2048} D^3$$

$$V_{TC} = (3\pi/4) \times D^2 a \left(1 + \sqrt{2}\right) + \left(2\sqrt{3}\pi D^2 a/4\right) - \left(3\pi/4\right) \times D^3 (1 + \sqrt{2}) - (2\sqrt{3}\pi D^3/4)$$

$$V_{CU} = \frac{3\pi}{4} D^2 a - \frac{4}{4} \pi D^3$$

$$V_{CIR} = a' - \frac{\pi}{4} t^2 a + \frac{8\pi}{4} t^3$$

The pore size of the four structures can be expressed by Eq. (2):

$$t_{TC} = (2 - \sqrt{2}) a - D$$

The volume of the four structures can be expressed by Eq. (1):
In the design, first calculate the strut diameter when the cell structure porosity is 65%, and then calculate the value of the cell length a when the pore size is 650 μm when the strut diameter D is unchanged. All scaffold are printed using SLM technology. The 3D printing is carried out by the equipment (FS271M System) of Sichuan Farsoon Turing Additive Manufacturing Technology Co., Ltd. The machine was equipped with a 500 W laser with a spot size of 70 μm. The layer thickness and scanning speed are 30 μm and 300 mm/s respectively. After completing the SLM process, all implants were in argon at 800°C Heat treatment for 2h, then ultrasonic cleaning with ethanol and distilled water three times (15 minutes each time).

2.2 Characterization of scaffolds

The Micro-CT technique was used to analyze the structure of the porous titanium scaffold, and then the Mimics21.0 (Materialise's interactive medical image control system, Italy) software was used to three-dimensional (3D) reconstruct and measure the scaffold and its surrounding bone tissue. The parameters such as the pore size of the scaffold, strut size, porosity and surface area are included. The pore size, strut size, porosity and surface area of the scaffold were measured.

2.3 Mechanical property of scaffolds

The mechanical experiment is carried out according to the ISO-13314 standard. A rectangular scaffold (n=5, 10×10×12 mm) was used for the compressive strength experiment. The material universal testing machine (WDW-300) of Changchun Kexin Testing Instrument Co.Ltd. was used for mechanical experiments, as shown in Fig.2A. During the experiment, compression was performed at a speed of 1 mm/min. The elastic modulus (E) and compressive strength (σ) of the porous scaffold are obtained from the stress-strain curve of the material. The elastic modulus of the scaffold is calculated according to the maximum slope in the elastic region of the stress-strain curve. The compressive strength of the scaffold is calculated by the 0.2% offset method, as shown in Fig. 2B is the typical stress-strain curve of DIA.

2.4 Surgical procedure
In this study, a total of 24 adult New Zealand white rabbits (2.5-3.0 kg) from the Experimental Animal Center of Southwest Medical University were selected, regardless of male or female. The temperature of the breeding room is 24℃, and the humidity is 60%. Rabbits have free access to water and food. The experimental plan was approved by the Southwest Medical University Laboratory Animal Protection and Welfare Committee in accordance with international standards. Twenty-four rabbits were randomly divided into two groups according to the implantation time (6 weeks, 12 weeks). In each group, 12 rabbits with a total of 24 femurs were implanted with four different cylindrical scaffolds (n=6, φ 5×8 mm). Intravenous injection of 3% pentobarbital sodium (30 mg/kg) under general anesthesia, 0.5% lidocaine local anesthesia. After skin preparation disinfection, a 3cm longitudinal incision was made in both femoral condyles for surgery. Cut the skin and subcutaneous tissue to separate the muscles, and cut the periosteum to expose the femoral condyle(Fig. 3A). A hole with a diameter of 5 mm and a depth of 8 mm was drilled in sequence on the lateral side of the femoral condyle with a low-speed drill (Fig. 3B). When drilling holes, use physiological saline to reduce the temperature to prevent tissue necrosis caused by local high temperature. After the drilling is completed, scaffold is implanted (Fig. 3C) and the wound is sutured in turn. Three days after surgery, intramuscular injection of cephalosporin antibiotics was used for anti-infective treatment. At 6 weeks and 12 weeks after the operation, the rabbits were killed by intravenous air injection. The femurs were removed, washed with formalin fixed water, dehydrated with ethanol, infiltrated and embedded, and the hard tissue was sectioned into 200 μm sections using EXAKT E300CP hard tissue slicer. After grinding and polishing, slices of about 70 μm are made, and the slices are stained by HE staining method and observed under an optical microscope. Observe whether there is new bone tissue, microvascular and fibrous tissue inside the scaffold.

Fig. 3AExposure of the distal lateral condyle of the femur B defect 5mm in diameter and 8mm in depth was drilled from the lateral femoral condyle of the rabbit at low speed; C Implant the titanium scaffold into the bone defect

2.5 Micro-CT analysis

To assess the effectiveness of new bone formation, rabbit femurs implanted with porous scaffolds were scanned using micro-CT (Micro-CT100, SCANCO Medical AG). Scanning parameters are set as: X-ray source voltage 90kV; Beam current 200 uA; The scanning resolution is 17.2 μm. After scanning the sample, the projection is reconstructed and segmented into a binary image, and further analyzed with Mimics 21.0 (Materialise, Belgium). The internal space of the scaffold and bone tissue growth into the scaffold was defined as the volume of interest, and the bone volume (BV) and total pore volume (TV) were measured by Micro-CT for further detailed data analysis. By calculating the ratio of BV to TV(BV/TV), the BT/TV value with higher bone
growth performance was quantitatively evaluated, indicating that more bone had grown into the scaffold.

2.6 Histological evaluation

The fixed femoral condyle was dehydrated in ethanol, then embedded with methyl methacrylate, and a saw blade was used to cut a 50 µm thick section along the long axis of the cylindrical scaffold. After staining with 1.2% trinitrophenol and 1% acid magenta (Van Gieson staining), the images were observed under light microscope and obtained by fluorescence microscope.

2.7 CFD simulation

The permeability, velocity and internal velocity streamline of different scaffolds were evaluated by CFD. Due to the symmetry of the structure, only 2×2×2 units were used for analysis in order to save calculation time. Assuming that the fluid is incompressible, Navier Stokes equation [20] is adopted for calculation:

\[ \frac{\partial \rho}{\partial t} - \rho \nabla^2 \mathbf{v} + \rho \mathbf{v} \cdot \nabla \mathbf{v} + \nabla p = \mathbf{F} \cdot \nabla \mathbf{v} = 0 \]  

where: \( \rho \), \( v \), \( \mu \) represent the fluid density (kg/m³), velocity of fluid flow (m/s) and dynamic fluid viscosity (kg/m/s), respectively. \( \nabla \) is the del operator, \( p \) and \( \mathbf{F} \) represent the pressure (MPa) and forces (N).

The permeability \( K \) of the four structures is calculated by Darcy's law equation [21]:

\[ K = \frac{v \mu L}{\Delta P} \]  

where: \( v \), \( L \), and \( \Delta P \) represent the inlet fluid flow velocity (m/s), model length (m), and pressure difference (MPa), respectively.

Analyzed and calculated using Ansys Fluent software, CFD simulation model shown in Fig. 4. During calculation, the inlet velocity was set at 0.1 mm/s, the outlet pressure was set at 0, the fluid density was set at 1050 kg/m³, and the viscosity was set at 0.0037 kg/m/s[22]. The extra fluid domain above the structure was to avoid boundary effect.

![Fig.4 Schematic diagram of CFD simulation boundary conditions](image)
2.8 Statistical analysis

Analysis was performed using SPSS software (SPSS Inc., Chicago, II, USA). All the data were expressed as mean ± standard deviation and analyzed with the one-way ANOVA. In all cases the results were considered statistically significant with a p-value less than 0.05.

3 Results and Discussion

3.1 Characterization of porous titanium scaffolds

Fig. 5A is an enlarged photo of the 3D printed titanium scaffold manufactured by SLM for mechanical experiments. Fig. 5B is an enlarged photo of the four cylindrical titanium scaffolds manufactured by SLM. Fig. 5C is an image obtained after 3D reconstruction of the titanium scaffold. The printed scaffold has good consistency with the 3D reconstruction model, and the aperture and scaffold size are well controlled and uniform. Fig. 5D shows the measured scaffold surface area after 3D reconstruction of the four porous titanium scaffolds.

The bone scaffold used in this experiment adopts SLM technology [15, 23], through which complex 3D metal parts can be manufactured with good controllability and repeatability. Therefore, the printed entity conforms to the CAD model with minimal error, which is helpful for us to control the parameters of the porous structure and reduce the experimental error. At the same time, relevant studies have shown that porous Ti6Al4V scaffolds have good biocompatibility and
are conducive to cell adhesion and proliferation [24], which also ensures the safety of our porous titanium scaffolds implanted in animals. Table 1 shows the theoretical values of the structural parameters of the porous titanium scaffolds with four different topological structures and the actual values of the measured parameters after 3D reconstruction, including porosity, pore size, strut size and volume. The difference between the theoretical and actual structural parameters is small, which means that the scaffold printed is of high quality. Small means that the quality of the printed scaffold is high.

Table 1 Structural parameters of porous titanium scaffolds with four different topological structures

| Sample | Porosity (%) | Pore size (um) | Unit size (mm) | Volume (mm³) |
|--------|--------------|----------------|----------------|-------------|
| DIA    | 64.5         | 64.8±1.2       | 650            | 650±2.9     | 1.6         | 1.6±0.3       | 55.9         | 55.4±0.4     |
| TC     | 65           | 65.3±1.1       | 650            | 648±4.1     | 1.86        | 1.84±0.4      | 54.8         | 55±0.4       |
| CIR    | 64.5         | 64.8±1.2       | 680            | 678±4.8     | 2           | 2.0±0.1       | 55.7         | 56±1.2       |
| CU     | 65           | 65±1.1         | 660            | 663±5.9     | 1.2         | 1.23±0.1      | 55           | 56±0.6       |

A: Actual value; T: Theoretical value

3.2 Mechanical properties of scaffold

Fig. 6 shows the stress-strain curves of the four scaffolds. It can be seen from the figure that the scaffold of CIR structure is the lowest (49 MPa), which is caused by the fact that the strut is not homogeneous and the weakest in the middle part. The yield strength of the other three strut types is relatively close. As shown in Table 2, the elastic modulus and yield strength values of the four strut structures measured by the experiment are shown. Studies have shown that the elastic modulus of bone trabeculae ranges from 0.1-4.5GPa [25], and the yield strength of proximal tibia and proximal femur ranges from 0.56-55.3MPa [26]. In this paper, except the CIR structure, the yield strength of the other three structures all exceeded this range. The elastic modulus range of the four structures is 1.9-4.2GPa, which can also well match the elastic modulus of the host bone tissue. Therefore, the structure in this paper can not only build the elastic modulus matching the host bone tissue, but also ensure the scaffold has a high yield strength, which can be well applied in the bone tissue scaffold.
Table 2 Elastic modulus and yield strength of the four scaffold structures

| scaffold type | Elastic modulus (E/GPa) | Yield strength (σ/MPa) |
|---------------|-------------------------|------------------------|
| DIA           | 2.1 ± 0.8               | 106 ± 6                |
| TC            | 4.4 ± 0.3               | 107 ± 3                |
| CIR           | 1.8 ± 0.5               | 49 ± 2                 |
| CU            | 2.6 ± 0.1               | 96 ± 5                 |

3.3 Micro-CT analysis

The titanium scaffold was implanted at the distal end of the rabbit femur, and the bone scaffold was removed for Micro-CT after 6 weeks and 12 weeks respectively. The formation of bone in the scaffold was evaluated by Micro-CT. Fig. 7A is the 3D reconstruction image of the scaffold and new bone. As can be seen from the reconstructed images, the number of new bones in the scaffold gradually increased with the increase of time. At 6W and 12W, DIA, CU, TC, CIR and CIR were in sequence from high to low, which was consistent with the ratio between BV and TV (BV/TV) calculated by quantitative analysis in Fig. 7B. Within 6 and 12 weeks, the BV/TV of DIA was 15.2% and 23.1%, CU 13.7% and 19.1%, TC 11.4% and 18.3%, CIR 9.8% and 16.9%, respectively. Since we accurately controlled the porosity and pore size of the porous scaffolds, the most likely reason for this osteogenic difference was the pore shape. Bael [15] according to the in vitro experimental results, circular pore structure is more prone to pore blockage than non-circular pore structure. This may lead to the delivery of nutrients and oxygen inside the scaffold, affecting bone growth. This may be one of the reasons that CIR structure has the least new bone. The DIA consists of 16 struts of equal length, each with an Angle of 109.5, which is similar to the intertrabecular Angle of human cancellous bone measured by Natalie [27]. Therefore, this trabecular bone-like structure may be beneficial to bone growth. As we all know, bone scaffold porosity and void size have an important effect on cell adhesion, proliferation, differentiation and new bone formation.

![Stress-strain curves of four kinds of scaffold structures](image)
Fig. 7A Micro-CT reconstruction of the distal femur of rabbits after 6 and 12 weeks of titanium scaffold implantation. Silver white represents bone scaffold and yellow represents new bone; B Titanium scaffold was implanted in the distal femur of rabbits. *P < 0.05, **P < 0.001 compared with DIA.

3.4 Histological analysis

The images were sacrificed in batches at 6W and 12W respectively, and the histological sections of the bone scaffolds obtained were stained with dehydrated embedded sections. Bone growth was qualitatively analyzed through the bone growth in the section diagram, and the histological section diagram of the scaffold obtained after Van-Gieson staining was shown in Fig. 8. These section diagrams clearly showed the formation of bone tissue in the scaffold, and with the increase of time, the amount of bone tissue in the pores of the scaffold increased gradually. The maximum size of new bone mass was DIA and the minimum size was CIR. This is consistent with the qualitative and quantitative results obtained by Micro-CT in Fig. 9 and Fig. 10. Therefore, according to the results of the slices, we further verified the accuracy of the experimental results.
3.5 CFD analysis

The permeability and velocity of fluid flow in the internal structure of the scaffold have a great influence on the growth of bone tissue in the scaffold [28]. The fluid flow inside the scaffold can provide necessary oxygen and nutrients for the growth of cells. Excessive permeability is not conducive to the adhesion of cells on the surface of the scaffold, while too small permeability cannot provide sufficient nutrients [29].

Fig. 9A shows the pressure cloud diagram of the scaffold. The pressure gradually decreases from the highest point of the inlet to the lowest point of the outlet. For different structures, the pressure is uniformly distributed within the scaffold. Fig. 9B shows the velocity cloud map of the scaffold. For CIR and CU structures, the internal structure is relatively simple, so the velocity difference of the scaffold is not very large. For DIA and TC structures, due to their complex internal structure, there is also a big difference in speed [30]. DIA velocity cloud shows that the velocity on the side of the scaffold is significantly greater than the fluid flow inside the scaffold. This indicates that the DIA structure can accelerate fluid flow inside the scaffold structure, facilitating fluid flow to more areas of the scaffold. Fig. 9C shows the pressure difference $\Delta P$ at the inlet and outlet of the four structures. It can be seen from the figure that the pressure drop difference of DIA, CIR and CU structures is not very great, while the pressure difference of TC structures is more than twice as large. This indicates that the internal obstruction of TC structure has a large effect on the fluid, which is not conducive to the flow of fluid inside the structure, nor conducive to the flow of fluid through more areas inside the structure. Fig. 9D shows the permeability of the four structures, which is calculated according to Eq. 4. The permeability range of the proximal tibia is $0.467\times10^{-9}$ m$^2$ to $14.810\times10^{-9}$ m$^2$ [31]. The permeability range of the four structures in this paper is $3.8\times10^{-9}$ m$^2$ to $5.9\times10^{-9}$ m$^2$. Therefore, the permeability is in line with the requirements of bone tissue implantation.
Fig. 9 Fluid simulation results of four structures

Fig. 10 Velocity flow diagrams for the four structures

Fig. 10 shows the velocity flow diagrams of the four scaffold structures. It can be seen from the figure that the velocity streamline of TC CIR and CU structure is relatively simple and flows
through less areas inside the structure. Compared to the other three structures, the DIA structure has a low internal flow rate, and the lower speed allows cells to attach more easily to the surface of the scaffold, thus making it easier to grow bone in the body. The speed of the other three structures at the intersection of struts is significantly higher than that at the pores, which can promote the migration of cells to a deeper depth and also make the cells less likely to adhere to the surface of struts. So the DIA cells grew more than the other three.

Pore structure is an important parameter that affects bone scaffold. Pore structure plays an important role in the mechanical properties of cell migration and adhesion tissue formation and nutrient diffusion. There are many in vitro experiments on pore structure with different conclusions, and many studies are limited to cell experiments [23, 32-34]. Cell culture is static, while the human environment is dynamic. The size of bone defects, blood flow, local inflammatory response, osteocytes involved in bone formation, osteoblasts and various endogenous growth factors released by them, and mechanical stimulation all have an impact on bone growth [32]. Therefore, in vivo experiments are needed for further confirmation. In this study, four kinds of porous titanium alloy scaffolds with different structures made by SLM were selected, and four kinds of scaffolds were implanted at the distal end of rabbit femur to evaluate the growth of bone tissue in vivo, so as to explore the optimal pore structure of bone growth. Based on CFD, the permeability, velocity and flow trajectory of the scaffold structure were calculated. The combination of in vivo experiments and CFD reveals the causes of bone ingrowth in different structures, which provides a new theoretical basis for the design of bone scaffolds in the future.

Before the scaffolds were implanted in vivo, mechanical experiments were conducted on four scaffolds to measure the elastic modulus and yield strength values of the four scaffolds. According to related literature, the elastic modulus of trabecular bone is in the range of 0.1 - 4.5 GPa [25], and the yield strength of vertebrae, proximal tibia and proximal femur is 0.56 - 55.3 MPa [26]. The results showed that the elastic modulus of the four kinds of scaffolds was within the normal range, which could avoid the stress masking effect caused by the mismatch of elastic modulus. The yield strength of the four kinds of scaffolds could meet the strength requirements of orthopedic implants.

Through quantitative and qualitative analysis of new bone mass, we concluded that DIA structural bone growth was the best and CIR growth was the worst, which was further verified in hard tissue sections. Compared with the results of in vitro experiments conducted by Bael[15], circular pore structure is more prone to pore blockage than non-circular pore structure. One of the reasons is the decrease of nutrients and oxygen transport inside the scaffold, which affects bone growth. The second reason is that the fluid mechanics shows that the DIA structure can accelerate the fluid flow inside the scaffold structure, which is beneficial to the fluid flow to more areas of the scaffold. At the same time, the flow velocity inside the DIA structure is not large. The lower speed can make the cells more easily adhere to the surface of the scaffold. Only when the cells stick together can they proliferate further, and thus make it easier to grow bone in the body. However, the results of Bael et al. [15] showed that obtuse angle was more likely to cause cell clog than acute angle, and TC structure was second only to DIA structure in this paper. DIA struts have an Angle of 109 between them. According to its structural analysis, DIA structure should grow into a poor one, but in fact, the opposite is true. We believe that Bael research on bone scaffold Angle is limited to two-dimensional level, while DIA angle is measured in 3D level, so this theory is not completely applicable to DIA structure. At the same time, according to fluid
mechanics analysis, the pressure difference of TC structure is more than twice that of DIA, CIR and CU structure, indicating that the internal obstruction of TC structure is greater, which is not conducive to the flow of fluid inside the structure, and is not conducive to the flow of fluid through more areas inside the structure, so the oxygen and nutrients obtained will be relatively less. However, the internal structure of TC is more complex than that of CIR and CU, and the site of internal cell attachment is more than that of the latter two. At the same time, it can be seen from the velocity flow streamline of TC CIR and CU that the velocity streamline of the three structures is relatively close. The triangular hole seems to be conducive to cell proliferation and differentiation compared with the square and round structure, so the TC structure is better than the CIR and CU structure in bone growth. In summary, based on in vitro and in vivo experiments, we concluded that DIA had the best structural bone growth.

At present, there are many topological structures used in scaffold design. In this paper, four common structures are selected. The comparison of the results obtained is limited to these four structures. In addition, the simulation of blood flow by using computer fluid mechanics cannot completely replace the situation in vivo, and the data may be biased to some extent, so further accuracy is needed in future studies.

4. Conclusion

This study carefully prepared by selective laser melting method similar porosity (65%), and the aperture size (650 µm), the structure of four different kinds of porous titanium alloy scaffold, through the in vivo experiment combined with CFD analysis, combining with the existing research, the different structure of bone ingrowth of bone scaffold are analyzed in this paper we draw the following conclusion:

1. SLM printing can print high strength and low modulus bone scaffolds and has a good application prospect in orthopedics.
2. The pore structure has a great influence on bone growth. Among the four different pore structures, DIA structure has the best bone growth effect.
3. CFD analysis, the permeability, flow rate and flow trajectory of the scaffold structure were calculated. The results showed that the internal fluid velocity difference of DIA structure was the smallest and the fluid flow trajectory was the longest in the scaffold, which was conducive to bone growth.
4. This paper provides a new method for the research of porous scaffolds by combining computational fluid dynamics analysis and in vivo experiments, and provides a new basis for the design of future scaffolds.

Abbreviations
DIA: diamond lattice unit ; CFD: computational fluid dynamics; SLM: selective laser melting

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