In vivo testing of porous Ti-25Nb alloy serving as a femoral stem prosthesis in a rabbit model

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Abstract. The aim of the present study was to observe the performance of Ti-25Nb alloys with various porosities as femoral stem prostheses in a rabbit model, thus providing basic experimental evidence for the development of porous prostheses. The porous Ti-25Nb alloy prostheses were designed according to the morphology of the medullary cavity. These prostheses were placed into the femoral medullary cavities in 36 New Zealand white rabbits. Postoperative X-ray films, scanning electron microscopy (SEM) of the implant interface, energy-dispersive spectroscopy (EDS) analysis of the implant surface, pulling-out test and general observations were conducted. The specimens showed good biocompatibility; there was no obvious bone absorption in porous Ti-25Nb specimens with different porosities at different time points observed using X-ray films. Under SEM examination, calcium deposits were observed inside the pores and in the interface between bone and prostheses. The EDS analysis demonstrated that calcium deposits were present on the surface of the prostheses at the eight-week point postoperatively. The pulling-out test showed good bonding strength between bone and implant; after pulling out, the surface and inside the pores of the prostheses all presented bone mass. Porous Ti-25Nb alloy implants presents good biocompatibility as well as providing a biological fixation between the bone and implant. A porosity of 70% is more advantageous to the newborn bone ingrowth, combined with achieving a more solid bone-implant interface.

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Introduction

Researchers proposed the concept of 'osseointegration' in the 1960s, which laid the theoretical foundation for prostheses implantation (1). Osseointegration is the close connection between implant and bone in the body without soft tissue interval, and is a prerequisite for prosthesis stability, secure fixation and for the prosthesis to bear certain pressure (2). There are numerous factors that affect osseointegration, including biocompatibility, shape design, material characteristics, patient bone conditions, the application of bioactive molecules and surgical techniques (2). Among these, material shape design and characterization are the key factors that impact bone-implant binding capacity and strength (2).

Currently, the surfaces of artificial joint prostheses are usually treated with rough surfaces and hydroxyapatite coating in order to provide good integration of the bone and implant (3,4). However, this treatment limits the depth and scope on the combination of bone and implants (5-7). A porous implant design is speculated to an effective way to improve bone ingrowth into prostheses and enhance the combination of the range between bone and prosthetic surface so that aseptic loosening may be prevented and long-term stability of artificial joints can be obtained (8).

However, at present although several porous materials have been used, they exhibit a range of disadvantages. Titanium (Ti) has been extensively used for its excellent physical properties and biocompatibility (9). Though Ti has a porosity of 70%, which exhibited a plateau stress of 53 MPa and an elastic modulus of 3.4 GPa (10), its compressive strength is still lower compared with cortical bone. Certain other elements, such as aluminum, nickel, iron, vanadium and chromidium, can generate adverse biological effects due to the release of metal ions (11). By contrast, Ti, niobium (Nb) and tantalum (Ta) are believed to be nontoxic metals with good biocompatibility (12). Compared with Ta, Ti and Nb are lightweight and inexpensive (13). There is limited prior research on porous Ti-Nb binary alloys. In our previous studies, we have investigated the biocompatibility of porous Ti-25Nb *in vitro* (13). This study aims to investigate the characteristics of porous Ti-25Nb *in vivo* and observe the binding capacity and strength of bone-implant interface, thus

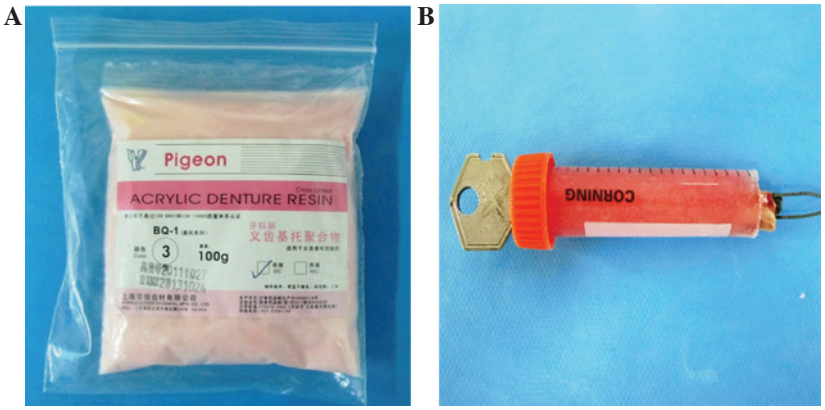


Figure 1. Specimens were prepared for the pulling-out test. (A) Denture powder was used for the fixation of the femurs with prostheses implanted. (B) A pre-prepared hole was designed to have a steel wire crossed into for the upper fixation to the clamp of the mechanical testing machine.

providing further theoretical basis for the clinical application of porous Ti-25Nb alloy.

Materials and methods

Ethical approval. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of Central South University (Changsha, China).

Design of the implanted prosthesis sample for the rabbit model. A New Zealand white rabbit, provided by the Experimental Animal Center of the Third Xiangya Hospital of Central South University, weighing ~2.4 kg was euthanized, a full-length femur was exposed after careful dissection, and then the anteroposterior and lateral X-rays were obtained. The femur was cut in the sagittal plane and coronal plane, respectively, so as to observe the morphology of the medullary cavity. The prosthesis specimen was designed accordingly.

Preparation of porous Ti-25Nb prosthesis samples. Samples with different porosities were prepared using powder metallurgy. The details of the process were described in our previous study (9).

In vivo experiment. A total of 36 healthy New Zealand rabbits (age, 4-6 months; weight, 2.2-2.6 kg) were divided into three groups (n=12 per group). Dense specimens (<2% porosity), specimens with 40% porosity and specimens with 70% porosity were tested in each of the three groups, respectively.

Surgical procedures. After the rabbits were anesthetized using pentobarbital (1 ml/kg; Sigma-Aldrich, St. Louis, MO, USA), skin preparation and sterilization were performed on the lateral sides of the left hips. A 3-cm straight incision was made on the skin of the greater trochanter; the subcutaneous tissues and deep fascia were cut layer by layer. Then, the gluteus maximus and the greater trochanter were exposed. Parts of muscles attached to the great trochanter were stripped, and the bone of the greater trochanter and the top of the femoral neck were broken away. The medial part of the femoral neck were carefully protected. Reamed into the medullary cavity, gradually enlarged it, and drilled into 2 cm depth using a drill 4-6 mm in diameter. The medullary cavity was cleaned using normal saline and specimens were implanted into the cavities along the longitudinal axis of the femur. The wound was flushed with hydrogen peroxide and saline and sutured layer by layer. Postoperatively, intramuscular injection of penicillin was performed to prevent infection, for three days.

Postoperative X-rays. Rabbits underwent anteroposterior and lateral X-rays at two, four, and eight-week time points postoperatively.

Sample extraction and preparation for tests. Three rabbits in each group were sacrificed using 3% pentobarbital overdose (4 ml/kg) at two, four and eight-week time points postoperatively. The bilateral femurs and soft tissue on them were all removed. Then they were immersed in formalin solution as saved backup. Two of the specimens in each group underwent scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS) analysis, with the rest for pulling-out test.

SEM of bone-implant interface and EDS analysis. The anterior and posterior femoral cortical bones were cut along the sagittal plane longitudinally, exposing the bone-implant interface. The specimens, which were to be observed by SEM and EDS analysis, were dried. EDS elemental analysis was performed to characterize the calcium (Ca), Ti, Nb and phosphorus (P) content of the samples.

Pulling-out test. The samples that contained femurs with porous Ti-25Nb specimens implanted were fixed into the mixture by mixing the denture powder (Fig. 1A) (denture base polymer; Shanghai Beiqiong Tooth Material Co., Ltd., Shanghai, China) with self-curing denture water (methyl methacrylate) in the mold. Care was taken to avoid the denture powder infiltrating into the interface of exposed prosthesis and bone, which may increase the pulling-out strength and affect the accuracy of the observed data. A steel wire was crossed into a hole pre-prepared on the prosthesis for pulling out (Fig. 1B), and it was fixed into the clamp of the mechanical testing machine (3369 Dual-Column Universal Testing System; Instron, Norwood, MA, USA). The distal ends of the specimens were also fixed. The machine's continual displacement of was set to

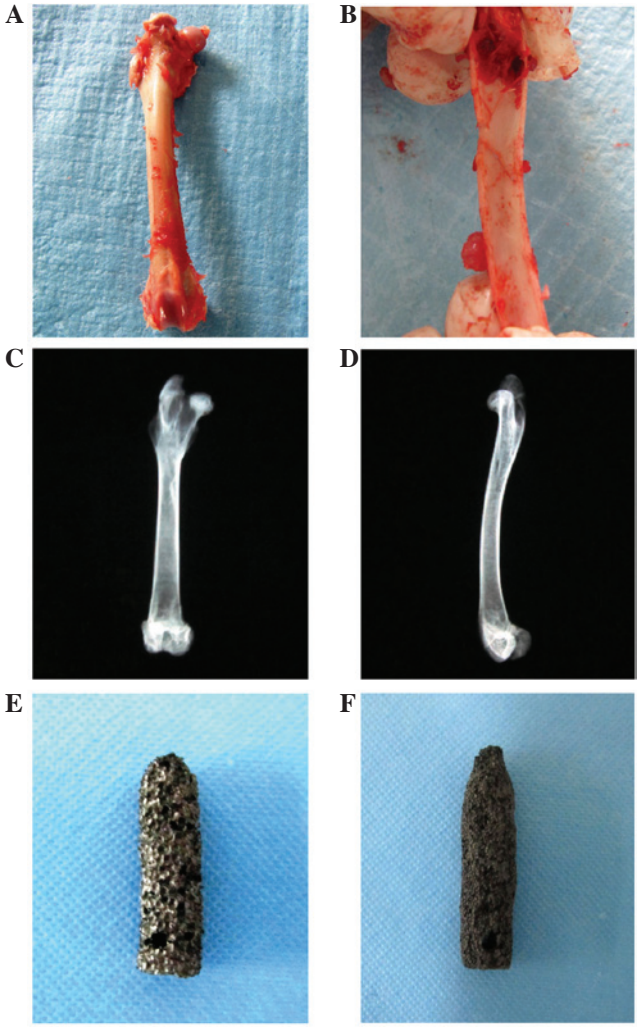


Figure 2. Design of the prostheses was based on the morphology, the inner diameter of the femur, and the X-ray observation. (A) The whole-length of the femur was exposed so that the morphology could be observed to inform the design of the prostheses. (B) The upper part of the femur was transected to observe the medullary cavity and measure the inner diameter. (C and D) Anteroposterior and lateral X-rays were obtained for the whole-length femur. The prostheses with (E) 70 and (F) 40% porosity were made as cylindrically shaped in the body with a diameter of 7 mm and a height of 25 mm, and its distal end turned out to be cone-shaped. A hole with a diameter of 1.5 mm was designed on the top part of the specimen so as to be prepared for the pulling-out test.

2 mm/min and underwent pretension for this test. The pulling strength and displacement were recorded automatically using a computer.

Statistical analysis. Statistical analyses were performed by SPSS version 20.0 (IBM SPSS, Amronk, NY, USA). Variance analysis was used to compare the differences between groups at the same time point. Values of $P<0.05$ were considered significant.

Results

Design of the implanted prosthesis. After dissecting the tissues around the femur, the morphology and the inner diameter of the medullary cavity were observed (Fig. 2A and B). The X-ray was obtained for the whole-length femur (Fig. 2C and D). It showed that the cortex of the lower femoral neck was thick, while the cortex in the femur shaft was loose. According to these characteristics, the prosthesis was designed for a cylindrical shape, with a diameter of 7 mm and a height of 25 mm. Its distal end turns out to be cone-shaped (Fig. 2E and F). A hole with a diameter of 1.5 mm was designed on the top part of the specimen so as to be prepared for the pulling-out test (Fig. 2E and F).

Observations for surgical procedures and postoperative conditions. The process of the surgical procedure of the specimen implantation was observed (Fig. 3). No fractures and side effects were observed during the operations and for seven days postoperatively.

Observations for gross specimens and HE staining. After the rabbits were euthanized, we dissected the tissues around to expose the implants. The results revealed no atrophy or noticeable secretions around the femurs (Fig. 4A). No loosening was observed in all the specimens (Fig. 4B-D). While the top part of the implant was exposed, a close bonding combination was seen between the bone and implant (Fig. 4D). The soft tissue around the top part of the implant was sliced for HE staining observation, and Ca was observed in the soft tissues around, and no obvious inflammation was seen (Fig. 4F).

X-ray examination. All specimens in each group showed a close connection with bone tissues. No low-density shadow of bone absorption was observed in bone tissues (Fig. 5A-K). By eight weeks, more cancellous bone was observed around the implants.

SEM observation. SEM showed Ca deposits on the surface of the materials (Fig. 6A and B). With regard to the bonding condition on the interface of bone and implant, gaps remained between them by two weeks (Fig. 6C and D). A close bonding was observed, with tissue ingrowth of the pores at the time point of four weeks (Fig. 6E and F). While it showed Ca deposits on the surface of the specimens, and more inside the pores by eight weeks, the interface between had no obvious boundaries (Fig. 6G and H). The sectional SEM showed osseointegration between bone and implant; bone ingrowth could be seen deeply inside the pores (Fig. 6I and J).

EDS analysis. By eight weeks, semiquantitative EDS analysis on the interface of a specimen and bone showed that Ca, Ti and Nb were 3.24, 23.26 and 3.53% (Fig. 7A), respectively, while inside the pores, 9.12% for Ca, 21.90% for Ti, 3.83% for Nb, and 4.37% for P was detected (Fig. 7B).

Analysis of pulling-out test. A pulling-out test was conducted using a mechanical testing machine (Instron) (Fig. 8A). The upper and distal ends of the specimens were fixed in one vertical axis so as to avoid shearing force. The data were collected automatically by the machine, linked to the computer. All tests were accomplished without any rupture of the prostheses; however, certain prostheses were well pulled out with part of the bone adhered (Fig. 8B and C). The data of the different groups are listed in Table I. At two, four and eight weeks, the maximum pulling-out force of the dense group was smaller compared with the other two groups, and the difference was statistically significant ($P<0.05$). The pulling-out force in the 70% porosity

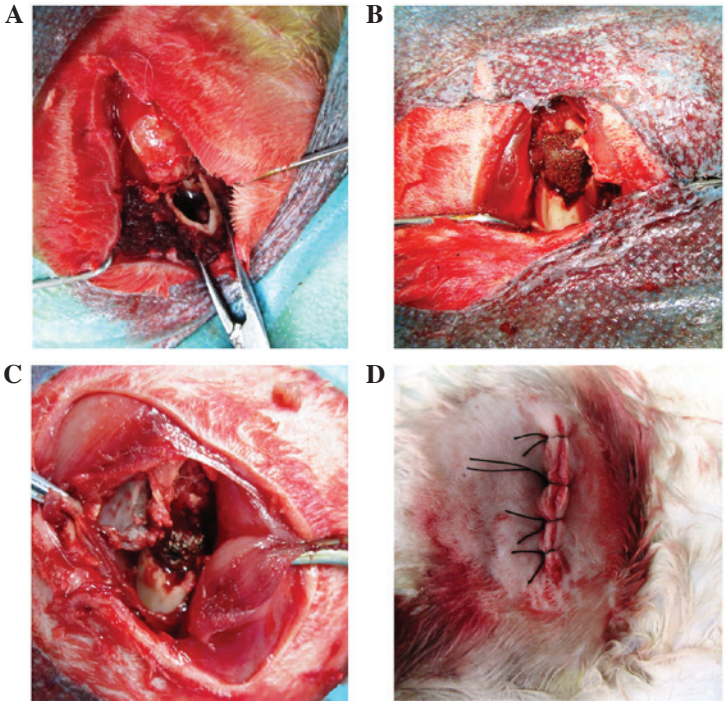
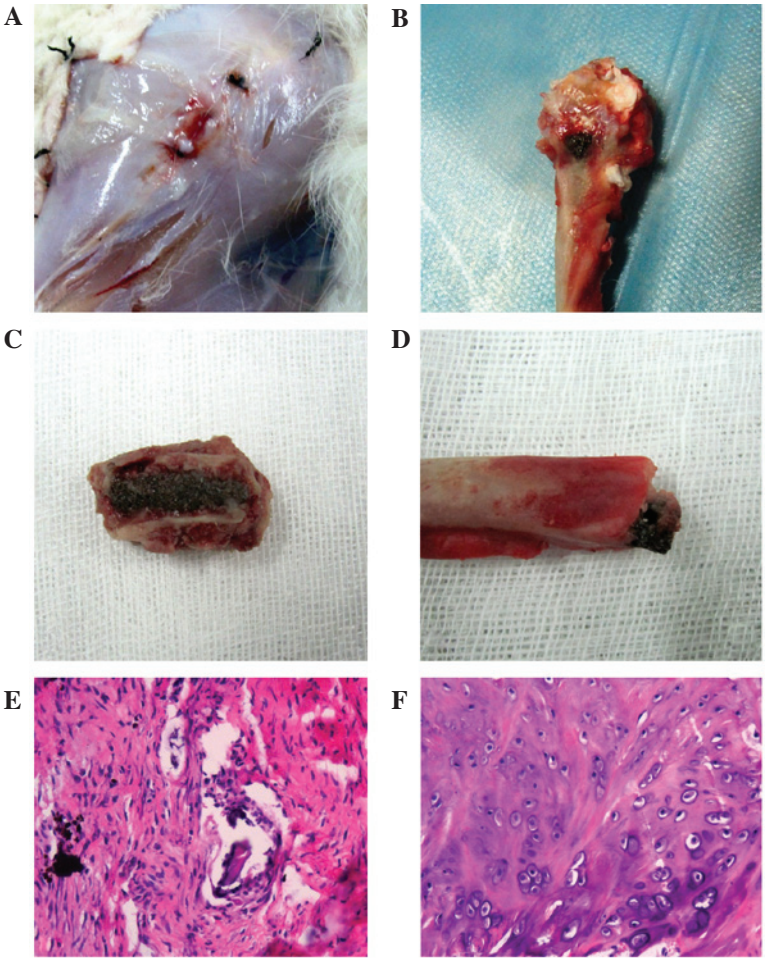


Figure 3. Surgeries were performed for the specimen implantation. (A) The proximal part of the femur was transected, and the medullary cavity was exposed. Prostheses of (B) 40 and (C) 70% porosity were implanted into the femur. (D) The wound postoperatively.



Figures 4. Rabbits were euthanized for four weeks postoperatively. (A) The original incision was exposed. No atrophy or secretions were observed around the femurs. (B and C) The prosthesis has a close bonding condition with the medullary cavity. No loosening was seen. (D) No rust was seen on the proximal part of the implant. (E) Calcium salt deposits could be seen in the soft tissues around the proximal part of the prosthesis (stain, hematoxylin and eosin; magnification, x200). (F) Osteoblasts were observed with no inflammatory response in the surrounding soft tissues (stain, hematoxylin and eosin; magnification, x400).

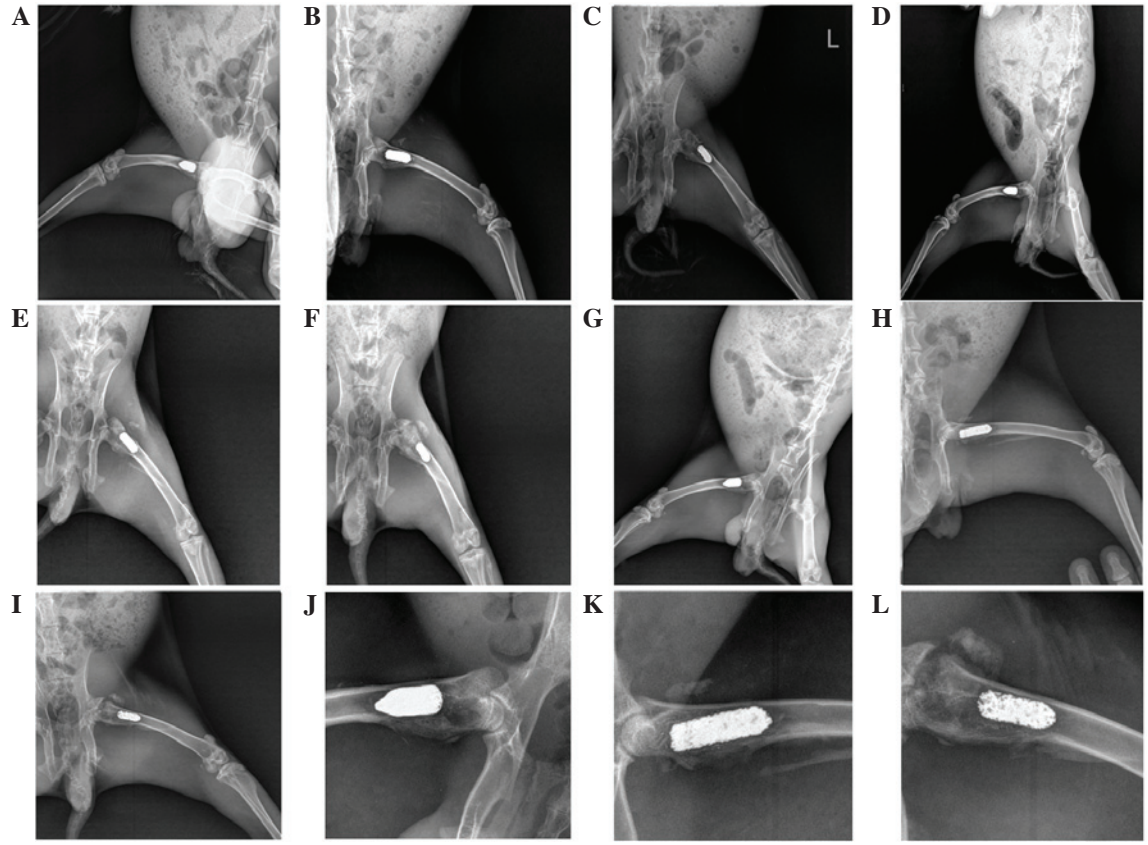


Figure 5. X-ray examination images from two, four and eight weeks postoperatively. (A) Dense prosthesis, (B) prosthesis with 40% porosity and (C) prosthesis with 70% porosity had been implanted for two weeks. (D) Dense prosthesis, (E) prosthesis with 40% porosity and (F) prosthesis with 70% porosity had been implanted for four weeks. (G) Dense prosthesis, (H) prosthesis with 40% porosity and prosthesis with (I) 70% porosity had been implanted for eight weeks. (J-L) Magnified X-ray images showed more cancellous bone around the implants by eight weeks (magnification, x10).

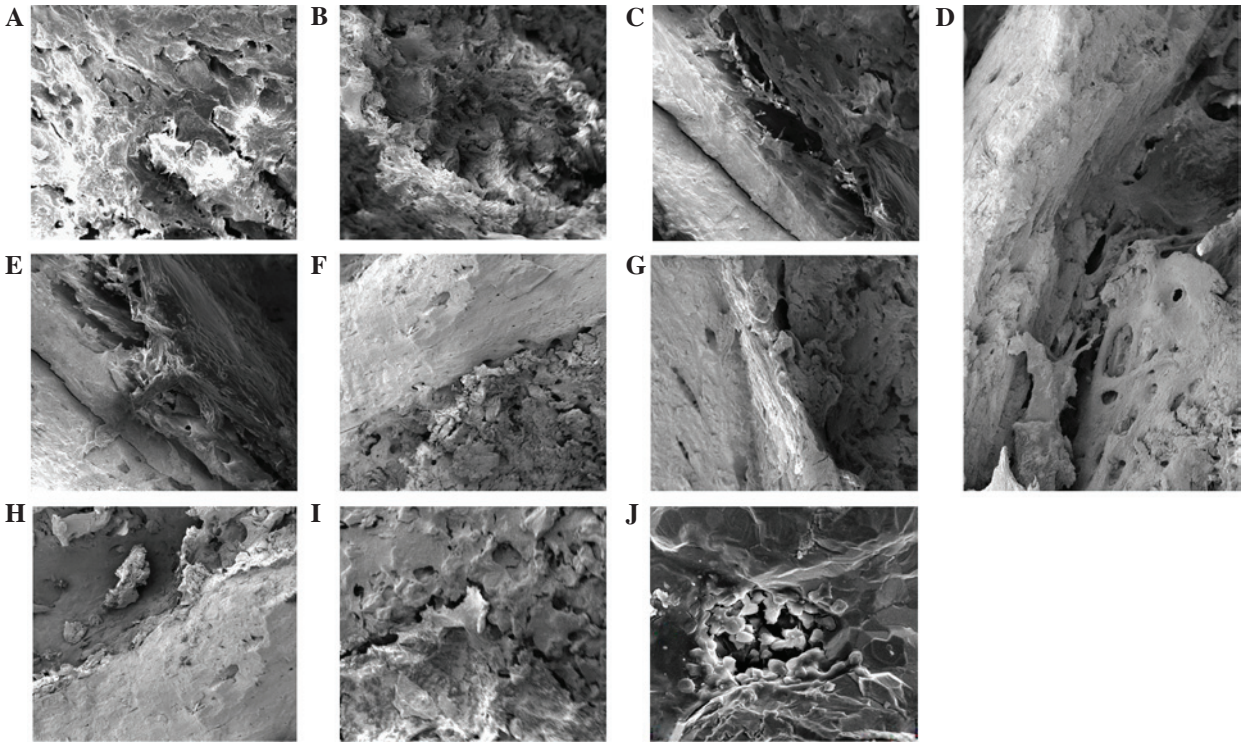


Figure 6. Results of scanning electron microscope (SEM) observation. (A and B) SEM showed calcium deposits on the surface of the materials. (C and D) The bonding condition on the interface of bone and implant were presented; gaps remained between them by two weeks. (E and F) A close bonding could be seen, with tissue ingrowth of the pores at the time point of four weeks. (G and H) While it showed calcium deposits on the surface of the specimens and more inside the pores by eight weeks, the interface between had no obvious boundaries. (I and J) The sectional SEM showed osseointegration between bone and implant; bone ingrowth could be seen deep inside the pores.

Table I. Comparison of pull-out strength in the three groups.

Group	Maximal pull-out force (N)		
	2 weeks	4 weeks	8 weeks
Dense	76.0±11.1	80.2±16.0	113.8±11.5
40% porosity	225.9±19.5	260.0±22.8	334.6±25.7
70% porosity	229.5±25.5	280.3±20.8	376.1±27.4

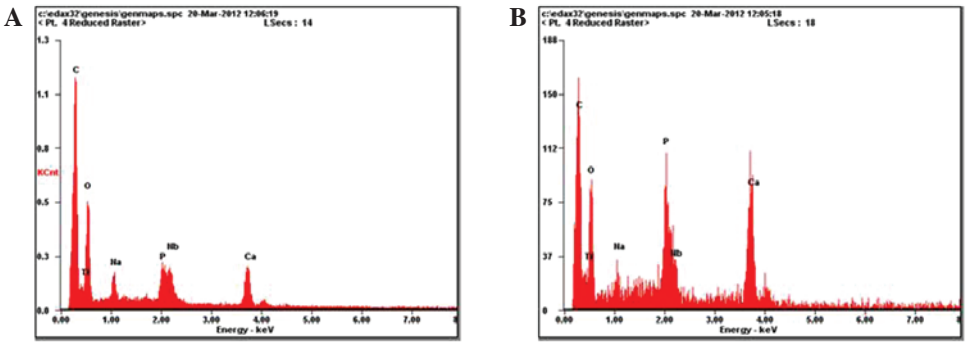


Figure 7. Energy dispersive X-ray spectroscopy (EDS) analysis. (A) By eight weeks, semiquantitative EDS analysis on the interface of a specimen and bone showed that calcium, titanium and niobium were 3.24, 23.26 and 3.53%, respectively. (B) Inside the pores, the values for calcium, titanium, niobium and phosphorus were 9.12, 21.90, 3.83 and 4.37%, respectively.

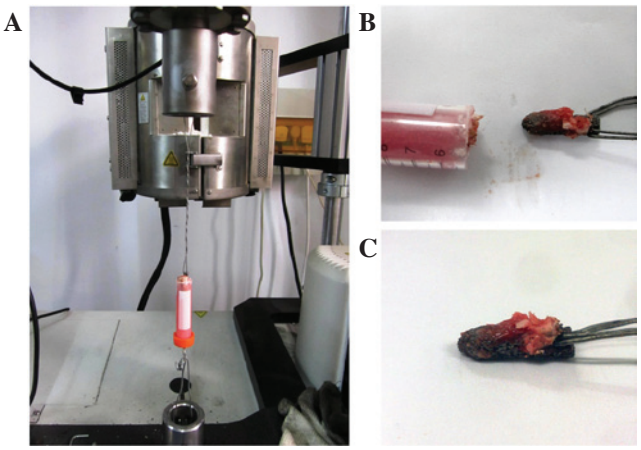


Figure 8. Analysis of pulling-out test. (A) Pulling-out test was conducted using a mechanical testing machine. (B and C) Certain prostheses were pulled out with part of the bone adhered.

group was significantly higher than the 40% porosity group by four and eight weeks (P<0.05), while there was no significant difference between the two groups at two weeks (P>0.05). By eight weeks, the maximum pulling-out force of the 40% and 70% porosity groups increased significantly compared with at two and four weeks (P<0.05), while the force between two and four weeks had no significant difference (P>0.05).

Discussion

Osseointegration is a phenomenon by which a prosthesis may make contact with the bone tissue directly and that is able to withstand the stress of use (1). It is a combinative reaction of bone and implant, which can be affected by a variety of factors, including biocompatibility of the implant, morphology design of the implant, surface structural properties and internal morphology of the implant, the partial mechanical environment and biological environment, systematic factors of the body, surgical techniques and the load supported (2). The bone-implant interface provides safe and reliable mechanical transmission between implants and bone tissue, and the situation of interface formation has a great influence on the long-term stability. A stable tightly combined interface is not only conducive to prevent migration of wear particles but also provides a good foundation for the long-term stability of the prosthesis (14). With the rate of the bone-implant interface osseointegration improved, the mechanical strength of the interface is also substantially improved and the early osseointegration has a greater impact on bone-implant interface strength (14).

The majority of the currently available Ti and Ti alloy implants in clinical application are dense (15,16). Although certain methods, such as surface treatment, can be applied to increase the contact area, these methods produce certain effects on bone integration between implants and bone. However, the bone tissue can only extend to the implant surface field and not the interior of implants (16). Biological fixation is not achieved when long-term stability is not guaranteed (16). Given the difference in mechanical properties, the dense-type alloy causes stress shelter after implantation in the body, which eventually leads to bone resorption. Numerous studies have indicated that excess bone loss influences the long-term effect of implants and leads to implant displacement, aseptic loosening, fracture around the prosthesis and increase in the difficulty of revision surgery (14-17). To improve the binding capacity between

interface of bone and implant, the concept of microtechnology was gradually introduced in recent years (18). Although several porous alloys are now available in the medical field, porous Ti-Nb alloy is a relatively cost-effective, which is also nontoxic and has good machinability and mechanical strength compared with other porous alloys (13,18). The porous Ti-Nb alloy used in the present study has a high strength with the elastic modulus body close to human cortical bone, which has been verified in our previous study *in vitro* (13). The degree of bone ingrowth of porous materials depends on several factors, including the porosity, the extent of micromotion and stability between implant and bone, and whether cortical or cancellous bone is contacted with the implant and the size of the gap between the implant and bone (19,20).

Furthermore, the porosity, pore size and bone-implant contact situation have effects on bone ingrowth, in addition to the spatial structure of the material key factor affecting bone conduction. Studies have shown that porous implants must have interconnected voids in order to provide space for the ingrowth of blood vessels and promote further bone tissue ingrowth (16,21). Another point is that the porous communication of the material is conductive to fluid transfer within the body, which can accelerate its tissue growth in order to increase the fixing strength (22). The presently investigated porous Ti-Nb alloys, which have a pore diameter of 200-500 μm, present with three-dimensional connectivity, thus can provide more space for ingrowth. The EDS analysis results in this study showed that Ca and P presented increased deposits in the 70% porosity specimens, which had more three-dimensional communication.

The increased surface area and roughness of the materials could improve the mechanical interlocking of the bone, thus enhancing the stability of the bone and implant. This mechanical interlock condition increased the strength of the pulling-out, while this condition could not be found in dense Ti-Nb alloys. Bonding strength depends on a combination area of bone-implant contacting, materials with higher porosity, and pore size that has a larger surface area; therefore, the capacity to resist pulling-out is increased (23-25). The pulling-out test showed that the strength was higher in the 70% and 40% porosity groups compared with the dense group after being implanted for four weeks. The strength for the 70% porosity group was significantly higher compared with the 40% porosity and dense groups after being implanted for eight weeks. This result indicates that high porosity provided more space for the ingrowth of the bone tissue and enhanced the mechanical interlock. De Vasconcellos *et al* (26) developed a porous Ti alloy using powder metallurgy technology, which can control the porosity, pore size, and pore connectivity. The *in vivo* experiment of his research also showed that high porosity, appropriate pore size and connectivity of porous material is good for bone tissue ingrowth and increase of pull-out strength.

SEM examination of the specimens after being implanted for four and eight weeks showed that the bone tissue grew into the inner pores. This was attributed to the properties of the trabecular bone that grows along the mechanical direction (27), which indicated that pores began bearing the mechanical load after they were implanted. This indirectly confirms the young modulus of this material, which is close to the bone around, so that it can avoid the shielding effect of stress. A study by Ryan *et al* (28) that the compressive strength and young modulus of the porous materials were more matched to bone rather than dense materials. The mismatch of the mechanical properties between bone and materials can cause stress shielding, leading to local bone resorption and low bone regeneration, thus affecting the growth of bone tissue. Studies have shown that the mechanical stability of the implants depends on the quantity of bone tissue present (28,29). The present experimental results showed that the pull-out force increased with the extension of the time after implantation. This may be associated with the increase of mature bone tissue around implants, which is consistent with the findings of Chen *et al* (30). As mentioned earlier, we altered the mechanical properties of the implant by adding the element of Nb into the Ti alloy, so as to more closely replicate the mechanical properties of bone. In addition, the porous Ti-Nb alloy not only improved the contact area of bone-implant and enhanced bonding strength, but also reduced the difference in elastic modulus between the bone and the implant.

In conclusion, a porous Ti-25Nb alloy implants presents good biocompatibility as well as providing a biological fixation between the bone and implant. A porosity of 70% is more advantageous to the newborn bone ingrowth, combined with achieving a more solid bone-implant interface.

Acknowledgements

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