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Enhanced Osseointegration of Porous Titanium Scaffold Implanted with Preload: An Experiment Study in Rabbits

Osteointegración Mejorada de Malla de Titanio Poroso Implantada con Precarga: Un Estudio Experimental en Conejos

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SUMMARY: Porous titanium alloy scaffold was widely used in treating bone defect caused by traumatic injury and osteomyelitis, which was incapable of self-healing. The implantation of scaffold produced stress shielding thereby forming osteolysis. The objective of this study was to analysis trabecular morphological features of osseointegrated bone. 14 New Zealand rabbits were divided into two groups, surgery group and healthy control group. 7 rabbits in surgery group were selected to perform 3D printed porous titanium alloy scaffold implantation surgery with preload at the defect of femoral condyle for osseointegration. The other 7 rabbits in control group were feed free. After 90 days healing, femoral condyles were extracted to perform micro-CT scanning with hydroxyapatite calibration phantom. Mean bone mineral density (BMD), bone volume fraction (BV/TV), BS/TV (bone surface area ratio), Tb.Th (thickness of trabeculae), Tb.N (number of trabeculae), Tb.Sp (trabecular separation) and DA (degree of anisotropy) were calculated from micro-CT images. The results revealed that osseointegration inside and at the surface of scaffolds worked well from grey values of micro-CT images. After 12 weeks healing, mean bone mineral densities (BMD) in surgery group and healthy control group were calculated as 800±20mg/cm3 and 980±90mg/cm3, respectively. This revealed that the strength of trabeculae in surgery group might lower than that in the healthy group. Trabecular morphological parameters test showed that trabecular morphological parameters at the surface of scaffolds in the surgery group deteriorated significantly. It was found from micro-CT images that ingrowth bone was filled with pores of scaffold. Overall, the effect of osseointegration was promoted through the change of mechanical micro-environment in the scaffold region. Overall, preload could improve osseointegration effect in the long-term after surgery. However, the trabecular morphology in the surgery group was deteriorated, which might bring secondary fracture risk again.

KEYWORDS: Osseointegration; 3D printed porous titanium alloy scaffold; preload; micro-CT; rabbit femoral condyle defect.

INTRODUCTION

Osseointegration was a research hotspot in treating bone defect, trauma, infection and bone tumor in the clinical and research field of orthopedics (Wang *et al*., 2017a). According to WHO report in 2015, about 75 million people suffered from trauma, in which 10 %-30 % victims were unable to walk (Paka & Pokrowiecki, 2018). Bone autograft was considered as a golden rule in bone defect repair (Dreifke *et al*., 2015). However, in most cases, it was impossible to acquire patient bone autograft during the treatment (Winkler el al., 2018). Nowadays, porous titanium alloy scaffold was widely used in clinical with its advantages of good mechanical properties, biocompatibility, and stable chemical composition (Torres-Sanchez *et al*., 2017).

Osseointegration, defined as bone ingrowth without interposed soft tissue, was found at bone to titanium screw implant contact surface (Carlsson *et al*., 1986). Osseointegration was found through microscopy after 3 months surgery. In recent years, 3D printing technology of titanium alloys had been developed and matured. With the introduction of 3D printing technology in prosthesis, the manufacture of porous titanium alloy scaffold became possible. In vivo studies of rabbit scaffold implantation, significant osseointegration and bone ingrowth ability of porous titanium alloy scaffold were found clearly through micro-CT images at four weeks after surgery (Chen *et al*., 2017). Meanwhile, integration of ingrowth tissue with

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scaffold could maintain good mechanical micro-environment and biocompatibility obviously (Wang *et al*., 2016).

It was found from in vitro and in vivo experiments that 3D printed porous titanium alloy scaffold could promote the effect of bone in-growth in the short-term after surgery. Osseointegration period could be shrunk and through structural optimization and surface modification before surgery (Salou *et al*., 2015; Srivasa *et al*., 2017; Ilea *et al*., 2019). In general, 3D printed scaffolds with high porosities and low stiffness were benefit for bone ingrowth compared with traditional implant (Wang *et al*., 2016). The porous scaffold fabricated with approximate diameter of 500 μm and porosity of 58 % by selective laser melting method showed significant bone in-growth as well as on-growth in rabbit femoral condyles after four weeks to eight weeks (Srivasa *et al*.). The effect of osseointegration was further improved after surface modification. The pores of implant were completely filled with bone within eight weeks in rabbit femur. The implant with anodized titanium dioxide nanotubes surface presented higher but not significant bone-to-implant ratio and bone surface area at the distance of 0.5 mm than acid-etched surface or machined surface (Salou *et al*.). The porous scaffold with mesh size of 800 um and coated by nano-hydroxyapatite promoted the new bone formation and improved the osseointegration process best at the defect region when compared with uncoated scaffolds (Ilea *et al*.).

The role of mechanical stimulation was increasingly recognized in the pathogenesis of peri-prosthetic osteolysis (Wang *et al*., 2017b). There were no significant differences on osseointegration effect between loading group and unloading group in the early time of postoperation (Reitmaier *et al*., 2018). In vivo mechanical stimulation could promote bone ingrowth into metallic scaffold to enhance long-term fixation after surgery (Willie *et al*., 2010). Implantation group coupled with the mechanical stimulation exhibited higher healing quality in contrast to the corresponding unloaded group during 4 weeks and 8 weeks (Zhang *et al*., 2018). Bone-to-implant surface area in loading group was about 10 % higher than unloading group at week 6, and more than 30 % at week 26 (Luan *et al*., 2019).

Limited literatures were available on mechanical properties of ingrowth bone tissue. Pull-out test could reflect trabecular mechanical properties of ingrowth bone tissue at the surface of oral implant (Ilea *et al*.). The Young's modulus of titanium alloy scaffold was significantly higher than bone tissue around. The stress shielding produced by scaffold broke original mechanical micro-environment resulting in abnormal bone remodeling In-growth bone tissue. Therefore, the mechanical properties of ingrowth bone tissue must be influenced.

The objective of this study is to compare trabecular morphological features after 90 days osseointegration in defect bone tissues with healthy trabecular tissues. All bone tissues were test using micro-CT after 90 days osseointegration. Trabecular morphological features in two groups were measured and compared at defect repair area. The change trends of trabecular morphological features were explored on ingrowth trabeculae long-term after surgery to provide applicable values for the improving mechanical micro-environment in clinical and bone tissue engineering research.

MATERIAL AND METHOD

Fourteen New Zealand rabbit with 2.5 months old and average weight of 2.5 kg were divided into two groups, including surgery group (7 rabbit) healthy control group (7 rabbit). 7 porous titanium alloy scaffolds were fabricated by 3D printer with the diameter of 2mm, the height of 5 mm, porosity of 70 %, pore diameter of 0.65 mm and wire diameter of 0.32 mm.

Rabbits in surgery group were anesthetized with 10 %w/v chloral hydrate solution. Intraperitoneal dose was calculated with 3.5 ml per kilogram weight. Regions around right condyles of femur were shaved clearly and skins were exposed. Limbs of rabbits were extended fixed on the operation table. 2 ml lidocaine hydrochloride with concentration of 0.02 g/ml was injected subcutaneously at knee joint for local anesthesia after skin disinfection with iodine. An incision of 4-5 cm was made at lateral knee using scalpel to exposed femoral condyle. A Small amount of lidocaine hydrochloride would be sprayed while waking up from anesthesia. Drilling location was set at the interosseous eminence center of right femoral condyle of rabbit. Drilling dimension was with the diameter of 1.9 mm and depth of 5mm. A sterilized 3D printed porous titanium alloy scaffold was implanted into drilling hole with preload produced by interference assembly. The implantation process was shown in Figure 1. The incision was sutured and disinfected with iodide. After waking up, rabbits were injected with enrofloxacin into the muscle of the operative leg with a dose of 0.2 ml /kg for three consecutive days. All rabbits were sacrificed at 90 days after surgery. The right femurs were extracted and frozen in -40 ºC freezer.

All femurs in two groups were performed micro-CT scanning. Micro-CT scanning parameters were pixel size 32 um, thickness 32 um, ROI 1500*1500 px, 1400 slices, voltage 190 kV, current 130 uA. All micro-CT images were imported into VG Studio MAX software to calculate

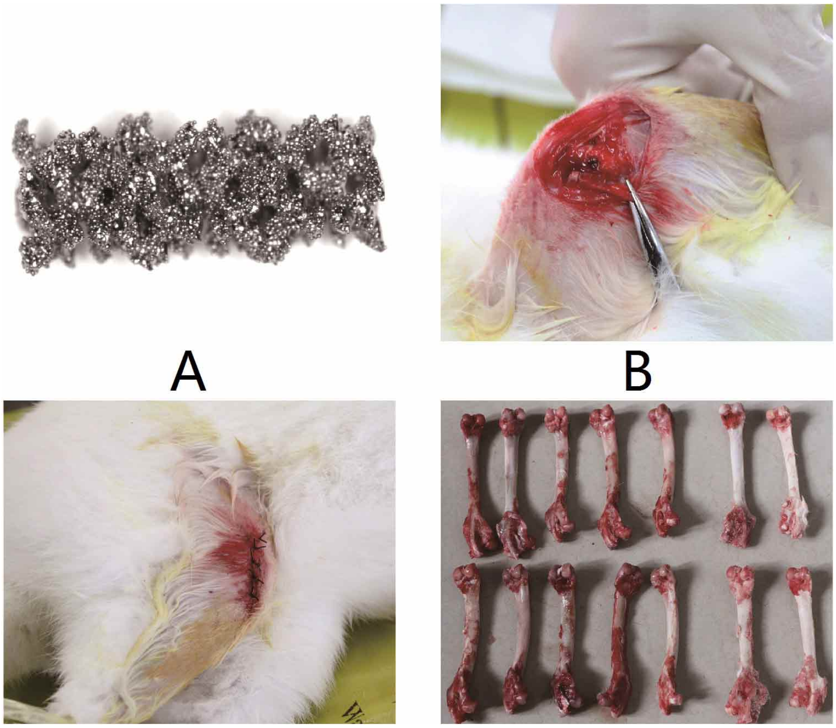

Fig. 1. Surgery process of scaffold implantation of a rabbit. A. 3D printed porous titanium scaffold. B. Surgery process of implantation. C. Stitching process after surgery. D. Right femurs from rabbits after 90 days osseointegration.

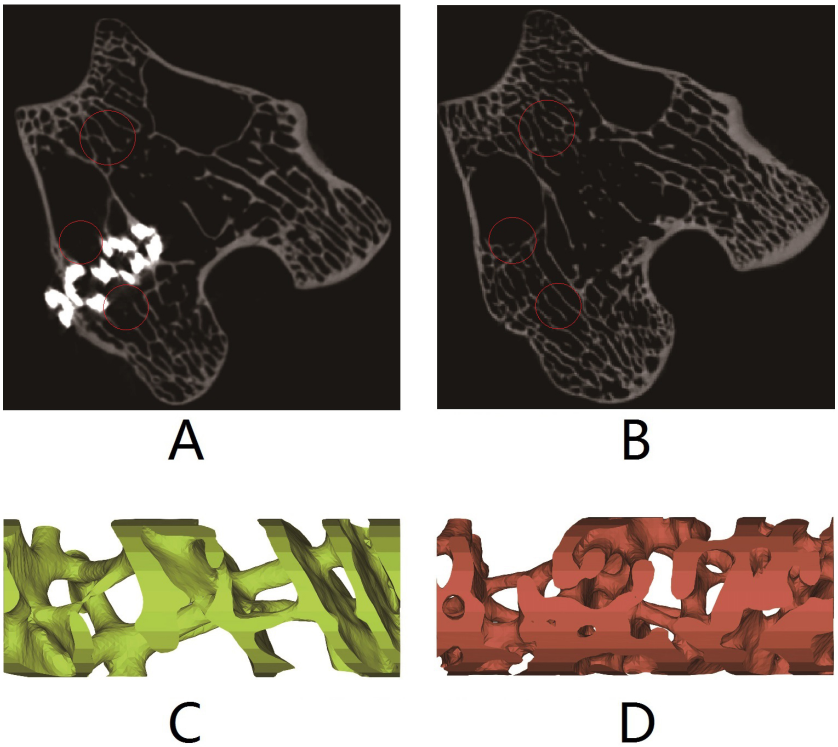

Fig. 2. Typical trabecular morphology comparison of surgery group and healthy control group. A. and B. Micro-CT images of femoral condyles from surgery group and healthy control group, respectively. C and D. 3D reconstruction models in the area of femoral condyles from surgery group and healthy control group, respectively.

trabecular morphological parameters at the region of scaffolds, including BV/TV (bone volume fraction), BS/TV (bone surface area ratio), Tb.Th (thickness of trabeculae), Tb.N (number of trabeculae), Tb.Sp(trabecular separation), DA (degree of anisotropy), BMD (bone mineral density).

All trabecular morphological parameters and bone mineral density data were expressed as an average ± standard deviation. Independent-sample T tests were performed on each trabecular morphological parameter and BMD with significance level of 95 %. Significant difference value P was considered of less than 0.05. All data were statistically analyzed and plotted in software SPSS 17.0.

RESULTS

Statistical analyses of trabecular morphological parameters revealed that there were significant differences between surgery group and healthy control group, which was shown in Table I as mean ± standard deviation. BV/TV, Tb.Th and Tb.N in surgery group were significantly lower than that in healthy control group. BS/BV and Tb.Sp in surgery group were significantly higher than that in healthy control group. There were no significant differences in DA between surgery group and healthy control group.

Typical micro-CT images and 3D reconstruction models in surgery group and healthy control group were shown in Figure 2. Significant differences of trabecular separation (Tb.Sp) were found in Figures 2a and 2b with the red circle markers. Three-dimensional reconstructions were performed on the micro-CT images. It was shown from three-dimensional models in Figures 2c and 2d that trabecular thickness (Tb.N) at the surface of scaffold in the surgery group was significant lower than that in healthy control group.

Table I. Trabecular morphological parameters of rabbit femoral condyle.

Groups	BV/TV	BS/BV	Tb.Th	Tb.N	Tb.Sp	DA
Group A	0.34±0.06	11.61±2.27	0.18±0.05	0.77±0.21	1.19±0.35	0.44±0.10
Group B	0.47±0.05	7.15±1.82	0.29±0.06	1.65±0.27	0.33±0.05	0.36±0.10
Difference test	0.000	0.006	0.008	0.000	0.002	0.224

$P<0.05$

DISCUSSION

This study focused on trabecular morphological features of osseointegrated bone tissues in the areas of porous titanium scaffolds. Rabbit femoral condyle defect model was established to perform osseointegration experiments. Bone defects, which caused by traumatic injury and osteomyelitis, were incapable of self-healing (McArthur *et al*., 2019). Porous scaffolds were widely used in treating trabecular bone defect to promoting cell proliferation, cell differentiation and bone ingrowth (Luan *et al*.). In our study, preload was exerted on the surface of scaffold using interference assembly with the interference of 0.1 mm. In the previous studies, it was found that scaffolds with porosity of 60-70 % and pore diameter of 100-400 μm could fit mechanical properties of trabeculae better and were more suitable for osseointegration (Liang *et al*., 2019). Our results of rabbit osseointegration experiments revealed that porous scaffolds with porosity of 70 % and pore diameter of 650 μm promoted bone ingrowth effectively after 12 weeks healing.

Although scaffold had good osseointegration ability and osteogenic induction ability, osteolysis were still found from 20 % revision surgeries (McArthur *et al*.). The main reasons for osteolysis were stress shielding caused by scaffold. In our study, the introduction of scaffold could still bring stress shielding, which could lead to osteolysis and high secondary fracture risk in the long-term after surgery. The statistical results showed that the significant reduced parameters were bone volume fraction (BV/TV), trabecular thickness (Tb.Th), number of trabeculae (Tb.N), and the significant increased parameters were trabecular surface ratio (BS/BV), trabecular separation (Tb.Sp) compared with healthy control group. These parameters all showed that the trabeculae deterioration trend at the surface of scaffolds. Peng *et al*. (2016) used commercial implants with resorbable blasting media treated surfaces to perform osseointegration on New Zealand rabbits. It was shown that bone volume fraction (BV/TV) was about 38 % (Peng *et al*.). This result was basically consistent with our data in surgery group, and less than healthy control group. Therefore, it could be concluded that both preload and surface modification could promote osseointegration effect. The better effect of osseointegration would be obtained if these two methods were combined together. Li *et al*. investigated the enhanced osseointegration of the scaffolds coated with graphene (Li *et al*., 2018). The BV/TV in the graphene coated group was 33.548 ± 2.678 % and the BV/TV in the uncoated group was 24.911 ± 2.898 % at 12 weeks. These results were all lower than our surgery group with preload. Therefore, mechanical micro-environment was an important factor in promoting bone remodeling process in the mid-long-term surgery.

Bone mineral density is a mainly noninvasive test to analyze bone strength. Micro-CT HA phantom was scanned with the same scanning parameters as bone micro-CT images to calibrate bone mineral density of rabbit femoral condyle. In our study, after 12 weeks healing, mean bone mineral densities in healthy group and surgery group were calculated as 980±90 mg/cm^3 and 800±20 mg/cm^3, respectively. Although the scaffold was filled with ingrowth bone tissue at week 12, trabeculae within the range of stress shielding was not as strong as healthy control group. Secondary fracture risk might occur in the long-term after surgery. Surface modification had already induced osteoblast differentiation in the early postoperative period. However, in the long-term after surgery, mechanical micro-environment was necessary for bone remodeling.

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LYU, L.; JING, Y.; WANG, J. & ZHANG, C. Osteointegración mejorada de andamio de titanio poroso implantado con precarga: un estudio experimental en conejos. *Int. J. Morphol., 38(4)*:909-913, 2020.

RESUMEN: La malla de aleación de titanio poroso se usó ampliamente en el tratamiento de defectos óseos causados por lesiones traumáticas y osteomielitis. El implante de la malla generó una protección contra el estrés, formando así osteolisis. El objetivo de este estudio fue analizar las características morfológicas trabeculares del hueso osteointegrado. Se dividieron 14 conejos (Neozelandeses) en dos grupos, grupo cirugía y grupo control saludable. Se seleccionaron 7 conejos en el grupo de cirugía para realizar una implantación de mallas de aleación de titanio poroso, impresas en 3D con precarga en el defecto del cóndilo femoral para la osteointegración. Los 7 conejos restantes del grupo control se mantuvieron sin alimentación. Después de 90 días de curación, se extrajeron los cóndilos femorales para realizar una exploración por micro-CT con un espectro de calibración de hidroxiapatita. Se calcularon a partir de imágenes de micro-CTDensidad mineral ósea media (DMO), fracción de volumen óseo (BV / TV), BS / TV (relación de área de superficie ósea), Tb.Th (espesor de trabéculas), Tb.N (número de trabéculas), Tb.Sp (trabecular separación) y DA (grado de anisotropía). Los resultados revelaron que la osteointegración dentro y en la superficie de los andamios funcionó bien a partir de los valores grises de las imágenes de micro-CT. Después de 12 semanas de curación, las densidades medias de minerales óseos (DMO) en el grupo cirugía y en el grupo control sano se calcularon como 800 ± 20 mg/cm^3 y 980 ± 90 mg/cm^3, respectivamente. Esto reveló que la fuerza de las trabéculas en el grupo de cirugía podría ser menor que la del grupo sano. La prueba de parámetros morfológicos trabeculares mostró que en el grupo de cirugía, la superficie de las mallas, se deterioraron significativamente. Se descubrió a partir de imágenes de micro-CT que el hueso en crecimiento estaba lleno de poros de andamio. En general, el efecto de la osteointegración se promovió mediante el cambio del microambiente mecánico en la región de la malla. En general, la precarga podría mejorar el efecto de osteointegración a largo plazo después de la cirugía. Sin embargo, la morfología trabecular en el grupo de cirugía se deterioró, lo que podría traer un nuevo riesgo de fractura secundaria.

PALABRAS CLAVE: Osteointegración; Mallas de aleación de titanio poroso impreso en 3D; precarga micro-CT; Defecto del cóndilo femoral en conejo.

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