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Effect of beta-tricalcium phosphate/poly-l-lactide composites on radial bone defects of rabbit

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ABSTRACT

Objective: To explore the effect of β-TCP/PLLA scaffold in repairing rabbit radial bone defects. **Methods:** Thirty New Zealand rabbits were divided into β-TCP /PLLA group (group A), pure PLLA group (group B) and contrast group (group C) randomly. The rabbits were sacrificed respectively after 4, 8, 12, 24 weeks and the X-ray film was performed at the same time to evaluate the repair effect in different groups. **Results:** X-ray film showed there was uneven low density bone callus development in defect region after 4 weeks in group A. The defect region was filled with neonate osseous tissue completely during 12-24 weeks. X-ray score revealed that repair of bone defect results significantly better than group B and group C. **Conclusions:** The β-TCP /PLLA composite is capable of repairing radial bone bone defects. β-TCP/PLLA scaffold is significant because of rapid degradation ability, good histocompatibility and osteogenic action.

1. Introduction

Artificial bone graft material is one of the most popular topics of orthopedic research, allogenic decalcified bone matrix of biosources, synthetic polymers, bio-ceramic materials or other substances all have their disadvantages and can not completely meet the clinical requirements of bone scaffold. In order to prepared bone tissue engineering scaffolds which meet the requirements, we use composite materials to make up each other for the lack of their performance and reach optimum combination. In this study, based on the experimental research on repairing large segment of bone defects of rabbit with beta-tricalcium phosphate/poly-l-lactide composites, we try to understand the performance and the repair effect of large segment of simulated-bioactive bone substitute and explore the feasibility to repaire large segment of radial bone defects of rabbit.

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2. Materials and methods

2.1. Materials

A total of 30 male New Zealand white rabbits were selected, at the age of 6-8 months, weighing 2.5-3.5 kg, which were provided by the Experimental Animal Center of Suzhou University, Certificate of Quality No: 082007. β-TCP/PLLA scaffold was prepared by Composite Materials Research Laboratory, Suzhou University. The mass fraction of β-TCP was 20%, and the particle size of the β-TCP was about 500 nm. The PLLA material average molecular weight of 200 000, the specification of the plate was 2.5 mm×2.5 mm×4 mm, the PLLA material was cut into 2 mm long cylinder along the horizontal axis, and made into long segments of β-TCP/PLLA large segment of simulated-bioactive bone substitute. Ethylene oxide was sterilized.

2.2. Animal experiments

A total of 30 rabbits were randomly divided into three groups, with 10 animals in each group. The experimental group was implanted with β-TCP/PLLA scaffold, control group A with pure PLLA material block and control group B as the contrast group had no implantation. After intravenous anesthesia with 30 g/L sodium pentobarbital at 1 mL/kg, the animals were kept at the horizontal position, disinfected and draped in a sterile manner. After skin preparation, the skin was incised in the upper-middle region on the inside of the right forearm, spatium intermusculare was isolated and the radial was exposed. The radial was sawed off with wire saw and caused transverse fracture model. The bone was cut and the periosteum was 15 mm from the middle-superior segment of the radius, then they were treated after washing. It was sutured layer by layer, one cage for each postoperatively. Free activities were allowed, and they has muscle injection of penicillin 400 wu to prevent infection.

2.3. Index detection

Postoperative diet, activity, wound infection and material leakage were observed daily. Pain score were given according to the movements of the affected limb and contralateral normal limbs. Standards were as follows: If the actions of the affected limb and contralateral normal limb were the same, score was 0; if affected limb was in claudication and need to raise during action, score was 1; If the affected limb was with obvious lameness and need to raise during action, score was 2; If the affected limb was not movable, score was 3. On the operative day and in the 4, 8, 12, 24 weeks after operation, the X-ray film was performed to observe and evaluate the repair effect in different groups. Two experimental animals were sacrificed by air embolism at the corresponding observation time, the radial on the operation side was obtained. Bone mineral density of the callus in the defect area was detected with Lunar DPX-Ldual energy X-ray absorptiometer. Implantation material surface was cleaned up with the filter paper and then washed with double distilled water. The flexural strength, shear strength and torsional strength of the material were detected.

2.4. Statistical analysis

All of the data were analyzed by SPSS 13.0 statistics software, and the data are expressed as mean±SD values. One sample *t*-test was applied in the comparison between two groups. $P<0.05$ has statistical significance.

3. Results

3.1. General observation

All specimens survived after operation, and there was no wound infection or materials leakage. After the fracture, there was severe obstacle of the affected limb. Pain scores were gradually decreased with the healing of the fractures and the use of drugs. In 4, 8 and 12 weeks, pain scores of the experimental group and the control group A were significantly lower than the control group B ($P<0.05$). The pain scores showed no obvious difference in 24 weeks between them, which had no statistically significant difference ($P>0.05$) (Table 1).

Table 1
Pain scores after fracture of rabbits in different groups (mean±SD).

Groups	4 weeks	8 weeks	12 weeks	24 weeks
Experimental group	$1.9\pm1.0^{\triangle}$	$1.0\pm0.5^{\triangle}$	$0.2\pm0.1^{\triangle}$	0.1 ± 0.1
Control group A	$2.0\pm1.2^{\triangle}$	$1.2\pm0.5^{\triangle}$	$0.3\pm0.1^{\triangle}$	0.1 ± 0.1
Control group B	$2.8\pm1.3^{*}$	$2.5\pm1.1^{*}$	1.1 ± 0.6	0.6 ± 0.3

Compared with control group A, $^{*}P<0.05$, compared with control group B , $^{\triangle}P<0.05$.

3.2. Radiographic results

According to X-ray results, the defects were not repaired the day after operation, and the implant materials were not developed. After 4 weeks, the defects area in the experimental group presented heterogeneous low density callus development, the development showed progressive increasement. After 12 weeks of the operation, there was almost no difference between the callus density and the normal radial bone mineral density; After 24 weeks, there was a good connection and integration with the broken end. The callus formation progress was similar in control group A and the experimental group. And there was no new bone callus formation in Control group B after 24 weeks, the defect was not repaired. The results of callus bone mineral density in various stages are shown in Table 2.

Tbale 2

Bone mineral density measurement of the affected limb of rabbits (mean±SD, mg/cm^2).

Groups	4 weeks	8 weeks	12 weeks	24 weeks
Experimental group	0.12±0.03	0.18±0.04	0.24±0.07	0.34±0.09
Control group A	0.10±0.02	0.15±0.04	0.21±0.06	0.32±0.11

3.3. Materials testing results of the experimental group and the control group

The beta-tricalcium phosphate/poly-l-lactide absorbable rods bending strength of the experimental group was (152.70±2.65) MPa preoperatively, and the poly-l-lactide absorbable rods bending strength in the control group A was (150.50±2.65) MPa preoperatively. All decreased gradually with time, the bending strength were lower than the experimental group at each time point. The bending strength of the control group was lower than the experimental group 4 and 8 weeks preoperative and postoperative, but the difference was not statistically significant. The bending strength of the control group was lower than the experimental group 12 and 24 weeks postoperative (P<0.05). The shear strength of absorbable rods of the experimental group was (125.40±3.50) MPa preoperatively, and the shear strength of absorbable rods of the control group A was (122.30±2.47) MPa. There was no significant difference of the shear strength between the two groups preoperatively and 48 weeks postoperatively. And the shear strength of the control group was lower than the experimental group 8, 12, 24 weeks postoperatively (P<0.05). There was no significant difference in torsional strength between the experimental group and the control group preoperatively and 4, 8, 12, 24 weeks postoperatively (P>0.05).

Table 3

Materials testing results.

Index		Experimental group	Control group A
Bending strength (MPa)	4 Time (weeks)	140.70±2.36	132.20±2.37
	8 Time (weeks)	136.40±2.32	124.60±2.36
	12 Time (weeks)	122.00±1.65*	103.30±1.98
	24 Time (weeks)	83.10±1.36*	56.20±1.09
Shear strength (MPa)	4 Time (weeks)	120.80±3.30	116.20±3.27
	8 Time (weeks)	1 106.70±2.82*	98.50±2.32
	12 Time (weeks)	96.60±2.03*	84.30±2.21
	24 Time (weeks)	48.70±1.36*	38.00±1.65
Torsional strength (GPa)	4 Time (weeks)	4.60±0.17	4.60±0.06
	8 Time (weeks)	3.80±0.16	3.70±0.15
	12 Time (weeks)	3.30±0.23	3.20±0.22
	24 Time (weeks)	2.40±0.21	2.20±0.38

Note: Compared with the control group A, *P<0.05.

4. Discussion

The material in this experiment was of high strength, good compatibility and good degradability, and had no side effects. It has to absorbe the fracture fixation, and at the same time its degradation rate should be coordinated with the bone healing rate. The β-TCP /PLLA composite material was used in this study, that's because the β-TCP and PLLA are respectively alkaline degradation products and acidic degradation products, the neutralization of them can break the PLLA autocatalytic reaction cycle chain, which can reduce early degradation rate, thereby increasing the intensity of the initial fixation; In addition, another important reason for selecting this material is that it can reduce the incidence of aseptic inflammation, which can create a favorable microenvironment for the osteoblast proliferation and new bone formation[1,2]. The process of degradation and bone creeping replacement can be more synchronized.

Schmitz *et al*[3] made a preliminary definition for large segment bone defects, put forward the concept of critical-sized defect. The critical bone defect length is based on the diameter of the long bones. If the defect length reached 1.5 times of the diameter of the long bones, it can be considered as a critical-sized defect. Many scholars defined the defect length more than 1.5 times of the diameter of the long bones as large segment of bone defect, and can be used in animal models[4]. Some scholars believe that the dimensions of the middle of the radial bone defect (including periosteum) ≥ 1.4 cm was more reliable[5].

For rabbit radius bone defect model established in this study, the size of the middle defect of the radial (including periosteum)=15 mm, which is consistent with the model established by Niemeyer and Li[6-8]. Thirty male New Zealand white rabbits aged 6-8 month were selected to create the models. After the fracture, there was severe obstacle of the affected limb. But after the implantation of β-TCP/PLLA scaffold large segment of simulated-bioactive bone substitute, pain scores were gradually decreased with the healing of the fractures and the use of drugs, and the affected limbs were gradually reactivate. After 4 weeks, the defects area in the experimental group presented heterogeneous low density callus development. After 12 weeks of the operation, there was almost no difference between the callus density and the normal radial bone mineral density; After 24 weeks, there was a good connection and integration with the broken end, which fully showed that the composite cell transplantation has a good repair capacity of bone defects. There was no complete bone union of the control group. The experimental group significantly expressed the osteogenic potential and the capability of differentiation and proliferation, bone matrix secretion, calcium deposits and bone tissue remodeling of rabbit osteoblasts, which showed the bone union effect of Beta-TCP/PLLA was through the mechanism of bone formation. The experimental group significantly expressed the osteogenic potential and the capability of differentiation and proliferation, bone matrix secretion, calcium deposits and bone tissue remodeling of rabbit osteoblasts, which showed the bone union effect of Beta-TCP / PLLA was through the mechanism of bone formation. There was no significant difference of the shear strength and torsional strength of the materials between the two groups.

In summary, the mechanical strength of β-TCP/PLLA material can maintain for a long time, and the degradation rate is slow *in vivo*, which can meet the requirements for the fixation of fractures and bone tissue healing[9,10].

This study showed the β-TCP /PLLA composite were capable of repairing radial bone bone defects. The β-TCP/PLLA scaffold is significant because of its rapid degradation ability, good histocompatibility and osteogenic action.

Conflict of interest statement

We declare that we have no conflict of interest.

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