

計畫名稱：骨科複合材料-可吸收性生醫陶瓷管柱體內塞去礦化骨及生長因子複合材料(2/3)

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Abstract:

Autogenous bone transfer has been an important part of reconstructive plastic surgery. Presently, available technique have the disadvantages of limitation of available donor site, loss amount of donor tissue, and possibility of donor defect or deformity. In the present study, a vascularized bone graft was created and cultured in the groin area of the New Zealand rabbit. The cylindrical ceramic chambers with 15 mm in length, 6 mm in outer diameter, and 3 mm in inner diameter were prepared by the sintered porous $\text{-Ca}_2\text{P}_2\text{O}_7$ with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7$ 10H₂O addition. In the first group, the chambers impregnated with autogenous bone fragments and allogeneous demineralized bone matrix with volum ratio 1:1 were cultured in the rabbit groin area with saphenous vessels passing through. In the second group, the chambers were treated as the same procedures as the first group without saphenous vessels passing through. In the third group, the chambers impregnated nothing were also cultured in the groin area with saphenous vessel passing through.

After 2, 4, 6, 8, and 12 weeks of operation, the animals were executed with an overdose of intravenous pentobarbital. The viability of the osseous tissue in the chamber was evaluated by the histological examination, microangiograms, and fluorochrome incorporation for the three groups. The autogenous bone chips could survive and retain their osteogenic properties while packed into the sintered porous $\text{-Ca}_2\text{P}_2\text{O}_7$ (with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7$ 10H₂O addition) ceramic chamber and implanted into the rabbit groin area upto 12 weeks. However, even at the longest time periods, considerable amounts of dead bone were present in the chambers. In addition, we observed bone resorption in the three groups upto 12 weeks, which might be attributed to lacking of physiological stress. There were significant difference in new bone formation and osseous cells viability among the three groups. The prevascularized vessels and autogenous bone chips were both necessary for the new bone formation and osteogenic property in the chamber under such a heterotopic circumstance. The biodegradable ceramic used in this study were gradually absorbed and dissolved in the physiological environment. However, the degradation debris of the ceramic showed no harm and no injury to the new bone formation. These findings support the concept of creating a preformed vascularized bone graft to reconstruct segmental bone defects.

1. Introduction

Sintered porous $\text{-Ca}_2\text{P}_2\text{O}_7$ with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7$ 10H₂O addition ceramic has been reported osteoconduction, withstand sever mechanical loading, and optimum biodegradable ability in the physiological environment [6]. The autogenous bone chips and allogeneous demineralized bone matrix were reported that could transfer viable bone-producing cells within the graft to a new anatomic location [5-7]. If we design a $\text{-Ca}_2\text{P}_2\text{O}_7$ with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7$ 10H₂O addition ceramic chamber impregnated with autogenous bone chips and allogeneous demineralized bone matrix, the new generated bone could be stimulated and formed within the porous structure of

the ceramic, and slowly replace it by a form of creeping substitute” is an attractive goal with clinical relevance.

In the present study, the biodegradable ceramic chamber filled with autogenous bone chips and allogeneic demineralized bone matrix was prepared. The packed ceramic chamber would be positioned in the groin area with the saphenous vessels passing through the chamber longitudinally in contact with the packed bone fragments. By combining the tissue-compatible characteristics of the biodegradable ceramic with the osteogenic and osteoinductive capacity of autogenous bone and utilizing the vessel-sprouting capability of a transposed vessel, authors would like to investigate whether autogenous bone tissue and cells could be bone generation under such a heterotopic circumstance, whether autogenous bone cells, known to osteogenic after transplantation to a groin site, retain their viability if placed in contact with the biodegradable ceramic materials, and whether the ceramic material chosen in this study is histocompatible and noninjurious to the free tissues into which they are implanted.

2. Materials and Methods

2-1 Ceramic chamber and allogeneic demineralized bone matrix preparation

Ceramic chamber preparation [6, 11]:

5 g $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ powder was dissolved in the distilled water and well mixed with 95 g $\text{Ca}_2\text{P}_2\text{O}_7$ powder as a slurry condition. The well mixed slurry was then dried at 50 °C for three days. The dried cake was ground into powder form and sieved into a processing particle in a range of 40-60 mesh by the addition of binders and lubricants, which facilitate pressing and are burned off in a later firing process. The sieved particles were then compact into a stainless steel die under a hydrostatic pressure of 270 MPa. The cylindrical compacts measured 6 mm in outer diameter, 3 mm in inner diameter, and 15 mm in length were made. The sintered porous $\text{Ca}_2\text{P}_2\text{O}_7$ with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ addition ceramic chambers were cut into two halves along longitudinally central line of the chamber.

Allogeneic demineralized bone matrix preparation [7, 12-13]:

Allogeneic demineralized bone matrix was prepared from the diaphysis of donor rabbit femur, tibiae, humeri by acid demineralization in 0.6 N HCl for 72 hours and defatting in 70% ethanol for 72 hours. Dry matrix was ground to 50-250 µm using a mill and nylon mesh filters. Ten New Zealand white rabbits were used for this purpose.

2-2 Surgical procedure and implantation

All animals were anesthetized with an intramuscular injection of ketamine (35mg/kg) and xylazine (5mg/kg) mixture. General anesthesia was maintained by inhalation of oxygen, nitrous oxide, and halothane. Ringer solution was infused continuously through an intravenous catheter placed in the ear. Antibiotic prophylaxis with 400,000 units procaine penicillin and 500 mg streptomycin was administered 30 minutes prior to skin incision and was continued 2 days postoperatively. The animals were shaved dorsally over both iliac crest and medially in the groin area of the right leg. Small autogeneic bone fragments were harvested from the iliac crest and washed in isotonic saline. Allogeneic demineralized bone matrix, which has been soaked previously for one hour in normal saline, was well mixed with autogeneic bone fragments and then placed in the ceramic chambers.

The saphenous vascular bundle (consisting of the artery and vein) was exposed in the right groin and dissected free from the surrounding tissues. The saphenous vessels of the first group animals were elevated, and one-half of the ceramic chamber previously filled with autogeneic bone chips and allogeneic demineralized bone matrix was positioned beneath the vascular bundle. The second half of the ceramic chamber was placed on the top of the first half, permitting the vessels to pass through the enclosed chamber. A single silk suture was around the chamber to prevent it from opening and the silk was closed with 5-0 Vicryl sutures. Animals of the second group were treated as the previously described, but without saphenous vessels passing through the chamber. Animals of the third group also had the same operation with saphenous vessels passing through the chamber, but without bone chips and demineralized bone matrix inside the chamber. The rabbits were returned to their cages, where they were permitted unrestrained mobility. After 2, 4, 6, 8, and 12 weeks of operation, the animals were executed with an overdose of intravenous pentobarbital.

2-3 Measurement and Analysis

Fluorochrome bone labeling:

Fluorochrome bone makers were administrated to indicate characteristics of bone growth. Double labeling was performed in all rabbits with oxytetracycline (30mg/kg) and calcine green (35mg/kg) by means of subcutaneous injections which were alternatively injected for every other week after operation [7, 12].

Microangiography:

To confirmed blood flow in the vascular bundle, rabbits from the vascularized group were sacrificed for microangiography. Immediately after sacrificing the animals, the abdominal cavity was opened, and the abdominal aorta was exposed. The proximal portion of the aorta was ligated, and a Teflon catheter was inserted distally from the ligation and fixed tightly with silk thread. The tip of the catheter was placed at least 5 mm proximally from the bifurcation. An incision was made on the inferior vena cava, and heparinized saline at 37 °C was infused at a pressure of 150 mmHg. Infusion was continued until the toes of both legs became completely white. Then a 5% gelatin and 5% carmine solution at 37 °C was infused at 150 mmHg. The infusion was continued for about 30 minutes after toes became pink and backflow of the dye from the inferior vena cava was observed. Immediately after infusion, the implants (ceramic chambers) were harvested with surrounding soft tissues and were placed in ice-cold formalin solution (20%) [14-15].

3. Results and Discussion

In the first group (the chambers with saphenous vessels passing through were impregnated with autogenous bone chips and allogeneous demineralized bone matrix), the chambers were covered by thinner layers of granulation tissue and were less firmly anchored to the host tissue after 2 weeks of operation. A large number of small vessels were observed in fibrous tissue surrounding the bone fragments at 4 weeks. New bone formation was present on the surface of acellular bone fragments adjacent to the implanted vessels. At 6 weeks, osteocytes and new bone formation were more widespread, and bony union between bone chips was observed. Newly formed small blood vessels with a smooth muscular were often noted. However, wide acellular areas were noted in the center of the fragments (Fig.1). In addition, numerous osteoclasts were seen on the surface of bone. The bone infrequently demonstrated basophilic nuclear staining with the lacunae. However, minimal areas in the vicinity

of the saphenous vessels had intensely stained osteocytes. Osteocytes appeared to be more numerous in specimens although more than half their visible lacunae were empty. Areas with stainable osteocyte nuclei always appeared to be located along the peripheral rim of the osseous fragments, while the acellular areas were more centrally located. The osseous matrix in the cellular area showed a slightly less dense structure, taking the eosin stain differently than the acellular portions.

By 8 weeks, stainable osteocytes were more widespread, with viable bone elements outlining and sometimes bridging between the dead bone fragments. The new bone was attached toward the surface of the ceramic internal pores. Fluorescent microscopy demonstrated uptake of the last five bone labels administered, although calcine green was sparse. The fluorescence in periosteal specimens had more of the parallel appearance normally occurring in osseous tissue.

At 12 weeks, the chamber central hollow area was occupied with increased amounts of lamellar bone and fatty marrow tissue. The laminar bone formation appeared with very few plump osteoblasts on its surface (Fig.2a and Fig.2b). Most of the demineralized bone matrix has resorbed, and were replaced by loose adipose tissue or bone marrow tissue.

In the second group (the chamber without vessels passing through was impregnated with autogenous bone chips and allogeneous demineralized bone matrix), necrosis of some of the bone chips always occurred at 2 weeks after operation; frequently, only the fat spaces were retained peripherally. Small, well-defined resorptive lacunae were noted; these may represent osteoclastic activity. The osteocytic lacunae throughout the bone were empty at 4 weeks. At 8 weeks, large numbers of osteoclasts were still present at the periphery and resorptive bays representing phagocytosis process could be seen eroding the bone chips and demineralized bone matrix. At 12 weeks, there was a strong vascular response around the margin of the chip with early vascular ingress, but bone formation was not seen at the periphery of the bone chips and demineralized bone matrix.

In the third group (the chamber with saphenous vessels passing through was not packed with autogenous bone chips and allogeneous demineralized bone matrix), all the spaces were filled with fibrous fatty tissue, without evidence of new bone formation. In the experimental period, specimen of all chambers multinucleated giant cells lined the interface between the chamber and surrounding soft tissue. The presence of the giant cells suggested a foreign body reaction, and may be related to degradation or resorption of the ceramic. There was no large scale inflammatory reaction around the chamber; it was simply encapsulated within a sheath composed of elongated fibroblastic cells arranged in multi-layer .

5. Conclusion

The autogenous bone chips could survive and retain their osteogenic properties while packed into the sintered porous $\text{-Ca}_2\text{P}_2\text{O}_7$ (with 5 wt% $\text{Na}_4\text{P}_2\text{O}_7$ 10H₂O addition) ceramic chamber and implanted into the rabbit groin are upto 12 weeks. There were significant difference in new bone formation and osseous cells viability among the three groups. The prevascularized vessels and autogenous bone chips were both necessary for the new bone formation and osteogenic property in the chamber under such a heterotopic circumstance. The biodegradable ceramic used in this study were gradually absorbed and dissolved in the physiological environment. However, the degradation debris of the ceramic showed no harm and no injury to the new bone formation.

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