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Research paper

Experimental study of natural hydroxyapatite/chitosan composite on reconstructing bone defects ☆

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Abstract

Objective: To study the possibility of natural hydroxyapatite/chitosan composite on repairing bone defects. **Methods:**We developed a natural hydroxyapatite/chitosan composite that could be molded into any desired shape. The powder component consists of natural hydroxyapatite, which is epurated from bone of pigs. The liquid component consists of malic acid and chitosan. Operations were performed on the left tibias of 15 white rabbits to create two square bone defects. One of the defects was reconstructed with the composite, while the other was not repaired and used as a blank control. Three of the animals were killed at the end of 2 weeks, 4 weeks, 8 weeks, 12 weeks and 16 weeks respectively and implants were evaluated anatomically and histologically. **Results:**No apparent rejection reaction was found, except for a mild inflammatory infiltration observed 2 weeks after surgery. Fibrous tissue became thinner 2 ~8 weeks after surgery and bony connections were detected 12 weeks after surgery. The new bone was the same as the recipient bone by the 16th postoperative week. **Conclusion:**The hydroxyapatite/chitosan composite has good biocompatibility and osteoconduction. It is a potential repairing material for clinical application.

Key words: Hydroxyapatite; Chitosan; Bone defect; Biocompatibility

INTRODUCTION

A bone defect is always a difficult problem for surgeons in oral and maxillofacial surgery, reconstructive orthopaedic, craniofacial and plastic surgery procedures. There are many means to repair bone defects including vascularized bone graft, non vascularized bone graft, allograft, and so on[1-5]. However, each has its own shortcomings, such as donor site morbidity and donor shortage for autograft, immunologic response, transmission of disease, difficulty of procurement, premature resorption and endemic risk for allografts. Although numerous synthetic bone substitutes using metals, ceramics, and polymers have been developed to promote bone regeneration for several decades, there exists a lack of confidence in their biological performances, particularly related to long term, in vivo safety and efficacy[6]. As a result, over the last few years, many biomaterials have been suggested as bone substitutes.

Hydroxyapatite(HA) has been studied as a possible substitute material for hard tissue due to its high biocompatibility and osteoconductivity, and it has been used clinically as artificial bone and for dental implants substitute[7-10]. However, the clinical use of HA as a bone substitute has proved problematic. For example, it is difficult to prevent the dispersion of HA granules and mold the granules into the desired shape. Some researchers added water-soluble polymers such as polylactic acid, polyglycolic acid and sodium alginate into HA to improve the handling properties[11-13]. In State Key Laboratory of Department of Biomedical Engineering, Southeast University natural HA has been epurated and a pilot study for the preparation of nano-grade-powder

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has been conducted[14]. Based on the natural structure of bones, hydroxyapatite/chitosan composite(NHC) was prepared in our previous study. The MTT-Test showed that the hydroxyapatite/chitosan composite had no cytotoxicity in vitro[15]. In this study NHC was implanted to the tibial defects of New Zealand rabbits and its biocompatible, bioabsorbable and osteoconductive properties were evaluated.

MATERIALS AND METHODS

Experimental materials

Natural hydroxyapatite/chitosan composite was provided by State Key Laboratory of Department of Biomedical Engineering, SEU. We collected pig bones and removed the soft tissues. The bones were then calcinated at 850℃ for 2 hours and ground for 36 hours. The resulting material looked chalky and averaged 2.78 μm in diameter. It had CO_3^{2-} negative radix(***Fig.1***). We mixed 2.77g natural HA, 0.03g CaO and 0.04g ZnO and blended the mixture to uniformity. Chitosan(0.1g) was then taken up into normal saline and soaked thoroughly. Malic acid (0.1g) was put into the liquid mixture and dissolved completely. Next we put the HA mixture into the liquid and churned it up quickly to make a paste. After the powder and liquid components mixed together, the composite had a consistency that could be molded into the desired shape. It became solidified at room temperature after about 10 minutes. The plastic material after solidifying had an intension of 26.2 MPa and an abundantly porous structure(***Fig.2***).

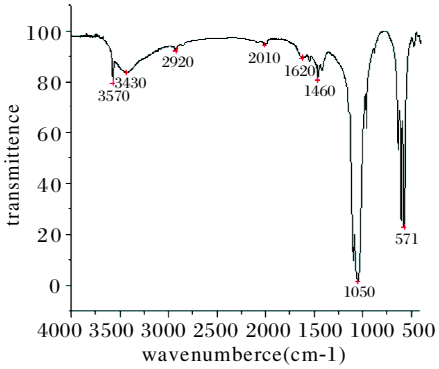

Fig. 1 IR spectra of natural HA

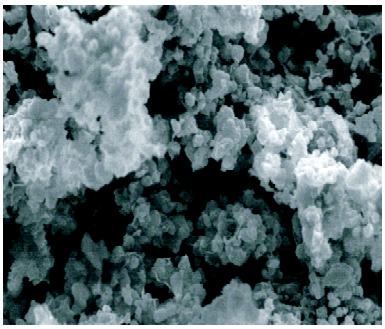

Fig. 2 SEM micrographs of porous hydroxyapatite/chitosan composite(SEM, × 4.00K)

Operation of the animal tests

Fifteen adult New Zealand white rabbits(average weight, 2.3-2.8 kg) were assigned to five groups:2-, 4-, 8-, 12- and 16 day groups. Each group consisted of 3 rabbits. After general anaesthesia with 20% ethyl carbamate(5 ml/kg), each rabbit was shaved and disinfected in an area over the left tibia. The tibia was exposed through a skin incision and two bone defects (6 mm in length, 4mm in width and 3mm in depth) were surgically created using saws and drills in the tibia of each rabbit. One of the defects was filled with NHC (experimental groups) before the composite had solidified, and the other defect was not filled and used as a blank control(control groups). The wounds were closed with silk threads. Cidomycin(1×10^5 U/Kg and 2 times/d) was injected into each rabbit for 3 days after the operation.

Microscopic observation and Histological study

Three of the animals were respectively sacrificed at the end of 2 weeks, 4 weeks, 8 weeks, 12 weeks and 16 weeks. The specimens were prepared for anatomical observation and histological sections. The materials were harvested with the surrounding tissue and fixed in 10% neutral formalin. Half of the samples were decalicified with 10% nitric acid, dehydrated, and then embedded in paraffin. These specimens were then sectioned at 7 μm and stained with Hemotoxylin-eosin(H-E) for histological observation.

RESULTS

Macroscopic observation

After the materials were implanted in the rabbit defects, all the wounds healed gradually and the rabbits were active with no postsurgical complications. No osteolysis, hyperplasia or other negative tissue responses were observed in the samples. The materials were not bare and were covered closely with soft tissue.

Histological observation

By direct observation, the implanted material existed as a block buried in the tibia. A mild inflammatory infiltrate was observed 2 weeks after surgery, but bone formation from the materials could not be identified (***Fig. 3***). New bone could be observed 4 weeks after surgery and high-quality bone bonding between the implants and host bone was observed. The voids between terminals of bone defects and implants, including the marrow cavities nearby, were filled with newly formed trabeculae with active osteoblasts. There was no evident dissolution at the edges of the implants. New bones could be observed at 8 and 12 weeks after surgery. From the sections retrieved at the end of post-operative week 12, partial direct contact between the trabeculae and the implant materials occurred. No

macrophages were found around the implants. The new bone was the same as the recipient bone and bony connections were observed at the 16th week following the operation(***Fig. 4***). However, obvious absorption could not be seen in the central body of the materials at that time. Thin new cancellous bones could be observed in the blank control groups in all periods.

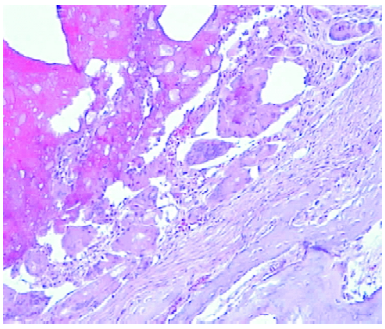

Fig. 3 Histological image of the experimental group 2 weeks after implantation(decalcified, magnification, HE, ×100)

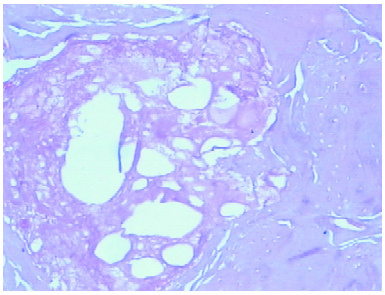

Fig. 4 Bone-implant interface of the experimental group specimen 16 weeks after implantation(decalcified, magnification, HE, ×100)

DISCUSSION

An ideal biomaterial for bone replacement materials should be amenable to contouring for optimal adaptation to the various shapes of bone defects, non-immunogenic and provide mechanical support when needed[6,16,17]. The autogenous bone graft shows most of these properties, but its limited availability and donor-site morbidity, along with its unpredictable rate and amount of remodeling and resorption has limited its use in the treatment of the bone defects. There are several alloplastic materials currently used in bone reconstruction, almost all of which are derived from sintered HA. Although sintered HA ceramics have good biocompatibility and osteoconductivity, they lack sufficient plasticity for easy molding into the desired shape. The new composite in this study has excellent handling properties and moldabilities. Thus, this material can be molded to fit the shape of even the most complicated bone defect and may be useful in oral and plastic surgery. This consistency is thought to be due to the addition of chitosan to the liquid component. Chitosan is a polysaccharide and a partially deacetylated chitin, which recently has been widely used as a biomaterial because of its biocompatibility[18-20]. Wang reported that the incorporation of phosphorylated chitosan and calcium phosphate cements showed excellent biocompatibility[21]. When using an organic acid in the liquid component of the cement, it is necessary to consider the inflammatory response induced by the acid in the early implantation stage.

In our study, a moderate inflammatory response was seen around implants 2 weeks after surgery. At the 16th postoperative week new bone had progressively formed on the surface of implants and incorporated with the host bone, showing that NCH has excellent biocompatibility. We think that the surfaces of natural HA particles have good bioactivities. When HA was implanted into the body mixed with CH, it increased the possibility for its active groups to interact with the body structures and formed an environment with high levels of calcium and phosphorus in the defects, which stimulated protein and osteoblasts to attach to the surface of the materials. This material may have the potential of inducing the mesenchymal stem cells (MSCs) to differentiate to the osteoblastic cells and promote their proliferation, which would be beneficial for the bone healing process. At the same time the biodegradation of NHC was slow. Composite could not be entirely bioabsorbed at the 16th postoperative weeks. That may be related to the contents of chitosan and the diameter of HA powder particles. In general NHC has good biocompatibility and osteoconduction and could be used as a repair material for bone defects.

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