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The repair of critical-size defects with porous hydroxyapatite/polyamide nanocomposite: an experimental study in rabbit mandibles

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Abstract. This study was conducted to evaluate the healing of critical-size surgical defects after implantation of porous nano-hydroxyapatite/polyamide composite (nHA/PA) blocks based on a bilateral mandible model using adult New Zealand white rabbits. 15 rabbits were divided randomly into three groups according to the observation period: 4, 12 and 24 weeks. The defects on one side were implanted with nHA/PA blocks and the contralateral defects were kept empty as blank controls. A combination of macroscopic, radiographic, histological and histomorphometric studies were performed up to 24 weeks postoperatively and compared with normal healing. Large amounts of callus and active osteoblasts were found in the pore structure after 4 weeks of implantation, and the defects were completely occupied by neo-bone with density comparable with that of host bone at 24 weeks. Significant difference was found between nHA/PA groups and blank controls regarding X-ray opacity over the whole period and bone parameters at 4 weeks postoperation ($P < 0.05$). The porous nHA/PA composite promotes bone formation over the extension of the defect, particularly in the early stage. Porous nHA/PA offers interesting potential for maxillofacial reconstructive procedures in load-free areas.

Key words: New Zealand rabbits; bone substitute; radiographic; histology; histomorphometry.

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The ideal bone-graft substitute should be an osteogenic, biocompatible, bioresorbable, easy to use material with specific properties that provide structural support and deliver a prescribed dosage of drugs for a stipulated time[1,16]. It is rare to find all these features in one material and composite materials seem to be the most suitable for clinical applications.

Hydroxyapatite (HA) has a composition and structure very close to natural bone mineral. HA can promote the formation of bone-like apatite on its surface[22], and is used in various biomedical fields.

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Polymers combined with HA can promote osteoblast adhesion, migration, differentiation and proliferation[23], which have potential applications in bone repair and regeneration. Polyamide (PA) has good biocompatibility with various human cells and tissues, probably because of its similarity to collagen protein in chemical structure and active groups[18]. PA also has excellent mechanical and corrosion-resisting properties resulting from the strong hydrogen bonds between the amide groups in PA macromolecules[12]. Particularly, as a polar polymer with high polarity, PA has a relatively high affinity to, and may form hydrogen bonds with, nano-sized apatite[23]. The biocompatibility and bioactivity of HA/PA composites has been confirmed in previous *in vitro* and *in vivo* studies[14,21].

Bioactive materials with interconnected porosity in their structure have added advantages in hard tissue prosthesis[5,19]. The porous structure of bioactive materials supports tissue in/on growth and supplements implant stability by biological fixation. The disadvantages of porous materials result from their low fracture strength and fatigue resistance. These materials could be used successfully in load-free situations or as coating on metal substrates[5].

In the present investigation, nano-hydroxyapatite/polyamide composite (nHA/PA) with high interconnecting porosity (65–75%) was tested as a bone substitute material in rabbit mandible defects. The biocompatibility of dense nHA/PA composite is excellent[14,21]. Porous nHA/PA was expected to promote bone repair in load-free maxillofacial defects. The purpose of this experiment was to evaluate the biocompatibility and osteogenesis of porous nHA/PA66 *in vivo* using a rabbit bilateral mandible defect (critical-size) model[13,15]. The relevant part of the mandible was collected 4, 12 and 24 weeks after surgery, to investigate bone healing by macroscopic, radiographic and histologic analysis.

Materials and methods

Nanocomposite characterization

The rectangular nHA/PA blocks (14 mm × 9 mm × 3 mm) were of high interconnecting porosity (65–75%); the pore size ranged from 300 to 500 μm (Fig. 1). The compressive strength for the block specimens was 3–7 MPa, similar to that of natural cancellous bone (2–15 MPa)[6,8]. The porous struts were initially pasteurized using distilled water and subsequently sterilized by gamma radiation (0.6×10^4 Gy, 6 min) prior to implantation.

Surgery

15 New Zealand white rabbits weighing about 3.5 kg were randomly divided into 3 groups of 5 animals each according to the observation period: 4, 12 and 24 weeks. Surgery was carried out under aseptic conditions and sedation by intravenous injection of 3% pentobarbital sodium (0.03 g/kg). A 2 cm incision was made approximately 1 cm lower to the lower edge of the mandible body to expose the bone. A bicortical critical-size defect (15 mm × 10 mm) was drilled at low speed under continuous saline irrigation on both sides of the mandible body in all animals (Fig. 2)[13,15]. The periosteum was removed during surgery to exclude the interference of periosteum osteogenesis. The nHA/PA blocks were inserted into the defects on one side of the mandible defect and the defects on the other side were kept empty. Implant placement was randomized to right and left sides and was secured in position by suturing muscle, subcutaneous tissue and skin in layers. All animals received intramuscular injection of penicillin 10,000 units per day immediately after surgery and continued for 3 days. The animals were killed by a lethal dose of barbiturate after a healing period of 4, 12 or 24 weeks. The mandibles were removed, cleaned of soft tissues and prepared for radiographic and histologic testing.

Local inflammatory reaction and would healing

Food intake, weight bearing, bone repair in terms of palpable callus, swelling, hematoma and associated signs of local inflammatory reactions were observed from the day of operation up to postoperative week 24.

Radiological examination

Radiographic examinations were performed immediately after surgery and after 4, 12 and 24 weeks using a dental digital X-ray unit (Siemens Heliodent, Siemens, Erlangen, Germany). The following parameters were standardized for all radiographs: projection distance of 10 cm, projection angle of 90° and exposure condition of 0.06 s at 4 mA. The degree of new bone formation during the healing period was estimated using the grey scale which is displayed automatically by the digital X-ray imaging system.

Histological study

Block samples were removed by cutting the mandible bodies about 8 mm away from the margin of the defects with a diamond saw. Decalcified sections of the bone samples were prepared to determine the status of the bone growth. The bone samples were washed thoroughly with normal saline and were placed in 10% formalin for 7 days. After initial fixation, the samples were dehydrated in a graded series of alcohol until the absolute was reached. They were decalcified and embedded in epoxy resin. Cross-sections of 5 μm thickness were cut at 200 μm intervals using a LEICA 2500E diamond saw microtome (Leica SpA, Milan, Italy) and were stained with Trichrome-masson.

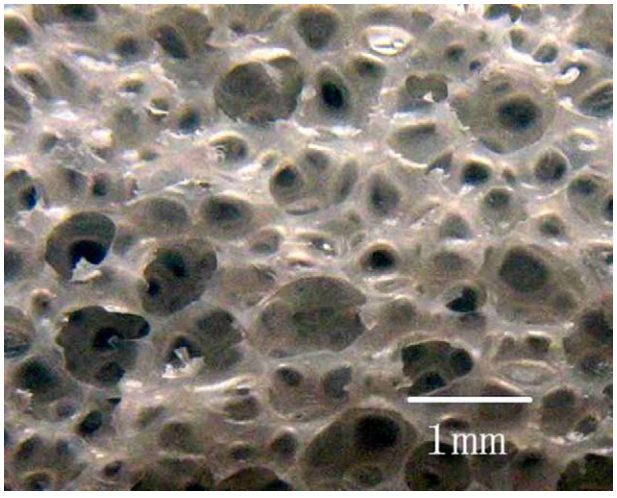

Fig. 1. Rectangular nHA/PA specimens before implantation.