Dynamic Analysis of New Bone Obtained by Nonvascular Transport Distraction Osteogenesis in Canines

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Purpose: The aim of the present study was to construct a nonvascular transport disc to repair the canine mandibular defects model and to perform a dynamic analysis of the new bone obtained by nonvascular transport distraction osteogenesis (NTDO) in canines.

Materials and Methods: Thirty adult dogs were randomly divided into 3 groups, with 10 dogs in each group. Canine mandibular defect models of NTDO were constructed. All the dogs were marked by tetracycline hydrochloride at a different distraction stage. The dogs were euthanized at 2, 4, and 12 weeks after distraction, and the quality ratio of calcium and phosphate for the new bone was measured using electron dispersive spectroscopy.

Results: The canine mandibular defects were successfully repaired. Using tetracycline hydrochloride, we successfully observed the quality and speed of new bone formation. The quality ratio of calcium and phosphate was similar between the new bone formation and the original bone. The time spent using a nonvascular transport disc to repair mandibular defects was consistent with using a vascularized transport disc, and the quality of the new bone and the original bone was exactly the same.

Conclusion: When the bone mass is insufficient or the conditions are not suitable for a vascularized transport disc, the nonvascular transport disc can be used as an alternative.

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Recently, a variety of methods has been used to treat bone defects, discrepancies, and deformities, including external fixators and the Ilizarov technique.[1,2] Transport distraction osteogenesis is usually used for skeletal reconstruction of large bone defects created by trauma, infection, tumor resection, and skeletal abnormalities in orthopedic and oral and maxillofacial surgery. Traditional theory has stated that enough blood supply for the transport disc is the key to successful distraction osteogenesis, usually requires enough soft tissue attached to the transport disc to ensure a sufficient blood supply source, and that it is difficult to obtain ideal osteogenesis by transport disc without a blood supply. Previous studies from our laboratory have shown that even without soft tissue attached to the transport disc, one can still obtain ideal osteogenesis.[3] However, some issues of bone repair and regeneration remain to be determined, including the quality of the new bone formation and how quickly it forms using nonvascular transport distraction osteogenesis (NTDO) and the differences, if any, in the composition between the existing bone and the new

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bone formation. Very little is known about these issues of bone repair and regeneration induced by NTDO. The purpose of the present study was to construct a nonvascular transport disc to repair a canine mandibular defects model and to perform a dynamic analysis of the new bone quality obtained by NTDO in canines.

Materials and Methods

DOGS

The animal ethics committee of Guangxi Medical University approved the present study. The Animal Experiment Center of Guangxi Medical University provided 30 healthy adult dogs, aged 1 to 2 years and weighing 10 to 15 kg. The right side of the mandible constituted the experimental group and the left side of the mandible was used as the control group.

MANDIBULAR DISTRACTION

Canine mandibular bone distractors were designed by the Zhongbang Company in Xian, China, according to the adult dogs' mandibular anatomic structure. The experimental dogs were anesthetized by intraperitoneal injection of 1/mL/kg pelltobarbitalum natricum (Shanghai West Tang Biotechnology Company, Shanghai, China). The mandibular operating area was prepared by removing the hair around the area of operation, applying antiseptic to the exposed skin, and administering local anesthesia. The mandibular body on the right side was approached with a submandibular incision, 1 cm below the inferior border. An area 3.5 cm long by 1.5 cm high was marked on the bony surface with a drill, 1 cm anterior to the mandibular angle. The anterior 1 cm of the marked area represented the osseous defect and the posterior 2 cm, the transport disk (Fig 1A). The ostectomy was performed with a drill and osteotomes (Fig 1B). The bony segments were removed from the surgical field and the 2-cm-long transport disk was fixated on the distraction device with 2 screws (Fig 1C,D). The device was then inserted and fixated across the bony defect with 2 screws on each side (Fig 1E). The activating arm was externalized through the skin anterior to the submandibular incision. One complete turn of the activating arm moved the transport disk by 1 mm anteriorly toward the defect. After surgery, the wound was sutured and flushed with gentamicin. From the first day postoperatively, the dogs were given an intramuscular injection of penicillin at 800,000 U (North China Pharmaceutical, Shenyang, China), twice a day for 6 days. Starting from the seventh day postoperatively, the distractors were initiated and activated at 1 mm/day, for a total of 10 days.

STATISTICAL ANALYSIS

The dogs in group 1 (n = 10) were euthanized at 2 weeks, those in group 2 (n = 10) at 4 weeks, and those in group 3 (n = 10) at 12 weeks after distraction. The transport disc, its surrounding tissue, and normal bone blocks of the left mandible at the same points as the right mandible were collected. The regenerated mandibles were marked by 5-tetracycline hydrochloride. Under the scanning electron microscope, the percentage of calcium and phosphorus of the contact surfaces between the transport disc and the new bone in all 3 groups and at 2 different parts of the new bone chosen randomly were measured by electron dispersive spectroscopy. The quality ratio of calcium phosphate was calculated. The results were statistically analyzed using the independent samples *t* test. Details of the operating methods are described in the subsequent sections.

SPECIMEN PREPARATION OF TETRACYCLINE FLUORESCENT TAGS

The cut size of each specimen was a 5-mm × 5-mm bone block placed in 70% alcohol and fixed for 3 days. Next, the specimens were dehydrated in gradient alcohol. Using a 3:1 mixing ratio, methyl methacrylate and dibutyl phthalate were mixed, adding to them the right amount of oxidation of benzene methyl. In a vacuum, the specimens were embedded into a 30-mm × 20-mm transparent cylinder. Slices without decalcification were made using a Leica 2600 sp hard tissue slicing machine (Leica Biosystems, Wetzlar, Germany), with a slice thickness of 5 μm. The slices were observed using an inverted fluorescence microscope, with a computer used to acquire the images.

SPECTROMETER COMPUTER ANALYSIS QUANTITY OF CALCIUM/PHOSPHATE RATIO

The canine mandibular transport disc and surrounding tissue specimens and the normal tissue of the control group in the same area were fixed with 2.5% glutaraldehyde liquid for 24 hours, with a phosphoric acid buffer wash 3 times, each time for about 15 minutes. Next, the specimens were placed into liquid nitrogen and frozen, at which they formed natural fractures. The surface of the transport disc and new bone tissue were tagged for observation. The cut size of the specimens was about 5 mm × 5 mm. They were placed in a phosphate buffer solution prepared using an ultrasonic cleaning machine wash for 10 minutes. Next, the specimens were dehydrated, dried, and prepared with mental spraying. The percentage of calcium and phosphorus was measured using electron dispersive spectroscopy to the contact surfaces between the transport disc and the new bone at different periods and the 2 different parts of the new bone.