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Nonvascular transport distraction osteogenesis in bone formation and regeneration. Is it an accidental phenomenon?☆

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ABSTRACT

Purpose: To explore the osteogenic mechanism of nonvascular transport distraction osteogenesis (NTDO) by constructing mandibular defects in dogs.
Methods: Sixty adult dogs were randomly divided into three groups with 20 dogs in each group. Canine mandibular defect models of NTDO were constructed. Animals were euthanized 1, 4 and 12 weeks after distraction, and the transport disc and surrounding tissue were collected and fixed. Histochemical staining using hematoxylin and eosin (H&E) and electron microscopic observations were used to examine bone regeneration.
Results: Distraction bone regeneration was observed in the distraction gap and around the transport disc, and osseous connections had formed between new bone and the transport disc after one week. Osteoclasts gathered around the transport disc, and bone absorption pit formation could be seen. After 4 weeks of distraction, the new bone around the transport disc was close to maturity with thick sclerostin on the middle of the transport disc. After 12 weeks the new bone and the transport disc were fully integrated, and were difficult to distinguish by H&E staining and electron microscopy.
Conclusions: Canine mandibular defects were successfully repaired by NTDO resulting in ideal new bone formation and fully recovered mandibular physiological function. The surrounding tissues, including musculoskeletal tissues, the periosteum and other soft tissues and the nonvascular transport disc, together contribute to bone regeneration and neovascularization in NTDO.

1. Introduction

Distraction osteogenesis is the biological process of new bone formation between the surfaces of bone segments that are gradually separated by incremental traction. Traditional theory believes that an adequate blood supply to the distraction site is critical for osteogenesis. Therefore, clinicians need to ensure that the soft tissues surrounding the site of the proposed distraction are well vascularized. However, during our clinical practice, we accidentally discovered that free autologous bone blocks, without periosteum and vasculature, could successfully be used in distraction osteogenesis. This new discovery challenged traditional distraction osteogenesis theory. To our knowledge there are no relevant research reports on nonvascular transport distraction osteogenesis. We constructed a canine mandibular defect model of nonvascular transport distraction osteogenesis to study this phenomenon and mechanism of osteogenesis.

2. Materials and methods

2.1. Animals

This study was approved by the animal ethics committee of Guangxi Medical University. The animal experiment center of Guangxi Medical University provided 60 healthy adult dogs, aged 1–2 years, weighing 13–15 kg.

2.2. Mandibular distraction

Canine mandibular bone distractors (Zhongbang Company, Xian, China) were designed according to the adult dogs' mandibular length, height and anatomical structure. Experimental dogs were anesthetized by intraperitoneal injection of Pelltobarbitalum Natricum (Shanghai West Tang Biotechnology Company, China) (1 ml/kg). The mandibular operating area was prepared by

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removing the hair around the area of operation, antiseptic skin preparation and local anesthesia. The exposed bone surface was prepared by cutting the mandibular segmental bone to a length of 3.3 cm and a height of 1.2 cm, and preserving the teeth of this area ensured that the channel between the wound cavity and oral cavity ceased to exist. The transport disc was made of bone block, of length 2 cm × height 1 cm × width 1 cm, which was cut from the mandibular bone defect area. The distractor was preset and fixed and the wound was sutured and flushed with gentamycin (Fig. 1). From the second day after operation, the dogs were given intramuscular injections of penicillin at 800,000 U (North China pharmaceutical, China), twice a day, for 7 days.

2.3. Analytical methods

Starting from the eighth day after surgery the distractors were initiated and activated at 1 mm/day, for a total of 10 days. Animals were euthanized 1, 4, or 12 weeks after distraction and the transport disc and its surrounding tissue were collected. The regenerated bone mandibles were assessed by scanning electron microscopy (model FEI Quanta 200 FEG, Philips, The Netherlands) and histochemical examinations using hematoxylin and eosin (H&E) staining.

3. Results

3.1. Health of animals

All experimental animals successfully completed the surgical procedure and survived to the scheduled time. Distractors were fixed firmly without any breakage or accidents during the experimental process. Wound infection was not observed in the experimental animals and the wounds healed well.

3.2. Specimen observation

The transport disc was found to be gradually covered by surrounding new bone starting from the lingual side, then from superior border, buccal side and inferior margin and was found to be fully integrated with new bone over time.

In the first week, the lingual side of the transport disc was completely covered by hard osteotylus-like tissue, while only a small area of the buccal side was covered by hard tissue. New bone was initially formed on the lingual side of the transport disc, which was then distributed around the transport disc and the distraction gap, with the thickest part located on the middle lingual side of the transport disc (Fig. 2). By four weeks, new bone tissue could be observed covering the surface on the buccal and lingual sides of the transport disc except on the inferior margin of the segmental transport disc (Fig. 3).

By 12 weeks the transport disc had completely fused with the surrounding new bone. It is hard to distinguish between the transport disc and new bone area with the naked eye. The whole distraction gap was filled with new bone tissue and mandibular continuity was fully recovered. The lingual bone surface was smooth, and the color of the new bone and its texture was the same as the original host bone. The buccal new bone surface was uneven, and the transport disc had no obvious boundary with the original host bone (Fig. 4).

3.3. Scanning electron microscopy results

Scanning electron microscope observations revealed new bone formation on the surface of the transport disc. We observed active growth of osteoblasts into the transport disc bone fiber gaps in the first week. The transport disc was surrounded by newly formed bone, and mature Haversian canal-like structures could be seen in four weeks. The cross-sectional shape of the new bone reached the level of normal bone and new bone matrix fiber, arranged along the horizontal distracting direction, was observed 12 weeks after surgery (Fig. 5).

3.4. Histochemical analysis

Osseous connection between new bone and the transport disc was visible when the distraction area was fixed after one week by H&E staining. New bone formation and large numbers of active fibroblasts and osteoblasts were observed around the transport disc. Many osteoclasts also gathered together on the surface of

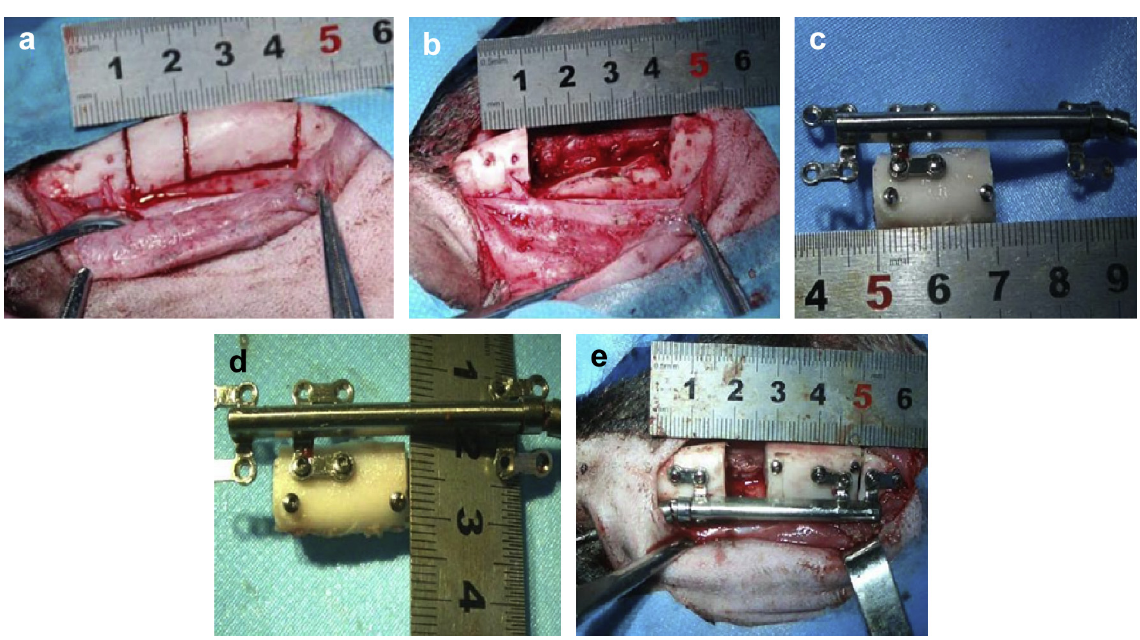

Fig. 1. a) Recording the marking; b) Bone defects caused by cutting bone; c) Manufacture of transport disc (length); d) Manufacture of transport disc (height); e) Installing distractor.