Local injection of nerve growth factor via a hydrogel enhances bone formation during mandibular distraction osteogenesis

Jian Cao, DDS, a Lei Wang, PhD, DDS, b De-lin Lei, DDS, c Yan-Pu Liu, PhD, c Zhao-jie Du, DDS, a and Fu-Zhai Cui, PhD, d Xi’an and Beijing, China
FOURTH MILITARY MEDICAL UNIVERSITY AND TSINGHUA UNIVERSITY

Objective. The objective of this study was to evaluate the effects of the injectable NGF-carrying collagen/nano-hydroxyapatite/alginate hydrogel on the bone formation in a rabbit mandibular distraction osteogenesis model.

Study design. Thirty-five New Zealand white rabbits underwent bilateral madibular distraction osteogenesis at a rate of 0.75 mm/12 h for 6 days. The rabbits were divided into 4 groups: group 1 received injections of collagen/nano-hydroxyapatite/alginate hydrogel containing hNGF; groups 2, 3, and 4 received injections of hNGFβ, Col/nHA/Alg hydrogel, and saline, respectively. The injections were performed on both sides of the mandible at the end of the lengthening phase. All the animals were killed at a consolidation time of 14 days.

Results. No difference in regenerate bone dimensions was observed among the 4 groups. Bone mineral density, the maximum load, and the bone volume/total volume of the new bone in the distraction gap in group 1 was significantly greater (P < .05) than in the other 3 groups.


Distraction osteogenesis (DO) techniques have been widely applied in the treatment of bone defects, deformities, alveolar vertical augmentation, and temporomandibular joint ankylosis in oral and maxillofacial surgery. However, a rather long duration of the bone consolidation phase is usually needed, which could contribute to many clinical complications and inconvenience patients. Therefore, the acceleration of bone formation and the compression of this duration will be beneficial clinically.

The nervous system plays an important role in bone metabolism and repair, and nerve growth factor is vital for maintenance and regeneration of nerves. In a rabbit inferior alveolar nerve defect model, an incidental observation revealed that administration of nerve growth factor (NGF) stimulated bone formation around the induced regenerating axons. NGF was also proved to be able to improve fracture healing in rats. In prior studies, we demonstrated that repeated injections of human NGF beta (hNGFβ) solution following the end of distraction could enhance the inferior alveolar nerve (IAN) regeneration as well as bone consolidation in a rabbit model of mandibular DO. Moreover, we hypothesized that the application of injectable delayed-release carrier materials could improve results by keeping NGF from inactivation and achieving a prolonged in vivo NGF concentration. Based on this point, we formulated a collagen/nano-hydroxyapatite/alginate (Col/nHA/Alg) hydrogel system, which not only has high osteoconductive activity, but also excellent injectability and sustained release ability.

The fabrication and properties of the collagen/nano-hydroxyapatite (Col/nHA) material was illustrated previously. In this study, we tested the effects of the injectable NGF-carrying collagen/nano-hydroxyapatite/alginate hydrogel on bone formation in a rabbit mandibular DO model.

MATERIAL AND METHODS

Animal model and grouping

Thirty-five skeletally mature (2.8-3.2 kg), male, New Zealand white rabbits were included. The animals were housed and cared for in accordance with the guidelines established by the Animal Center for Medical Experiment at Fourth Military Medical University. The details...
of the animal model were described previously. Briefly, a vertical osteotomy was performed bilaterally between the premolar teeth and mental foramen using a fissure bur. A titanium distraction device (Zhongbang Titanium Biomaterials Corporation, Xi'an, China) was fixed, with the distraction rod emerging into the labial vestibule (Fig. 1). Surgeries were performed carefully to avoid injury to the IAN. After a latency period of 3.5 days, gradual distraction was performed at a rate of 0.75 mm per 12 hours for 6 days.

Among the 35 experimental animals, 4 rabbits were killed before the completion of the experiment and excluded from the experimental groups because of soft tissue complications (n = 2) and accidental injury to the IAN from the chisel (n = 2). The remaining 31 rabbits were randomly divided into 4 experimental groups according to different injections percutaneously into the palpable callus: group 1 (n = 8) received injections of 0.2 mL Col/nHA/Alg hydrogel containing 20 μg hNGFβ (Laboratory of Biochemistry, Fourth Military Medical University, Xi’an, China); group 2 (n = 8) received injections of 20 μg hNGFβ in 0.2-mL isotonic saline; group 3 (n = 8) received injections of 0.2 mL Col/nHA/Alg hydrogel alone; group 4 (n = 7) received injections of 0.2 mL isotonic saline alone. The injections were performed on both sides of the mandible under general anesthesia at the end of the lengthening phase. Under radiograph guidance, the acupuncture point was 45 degrees to the skin and away from the IAN as described previously.
Preparation of Col/nHA/Alg hydrogel containing hNGF

The detailed synthesis techniques to fabricate a collagen/nano-HA composite (Col/nHA) were described previously.12-14 Col/nHA microparticles were mixed in purified alginate hydrogel (Biomaterials Laboratory, Department of Materials Science and Engineering, Tsinghua University, Beijing, China) with hNGF at a concentration of 0.1 mg/mL until homogeneously dispersed. All material handling occurred under sterile conditions. The injectable materials were ready for experiments before gelation.

Sample harvesting and radiographic examination

At consolidation time of 14 days, all the rabbits were killed. After carotid artery perfusion, internal fixation of the tissue was performed using 2.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1-M/L phosphate-buffered saline (pH 7.4). Each left hemimandible was harvested with the soft tissue excised. Plain radiographs were taken and the hemimandibles were scanned under a dual-energy X-ray absorptiometer (DEXA, Lunar DPX-1Q, Lunar Radiation Corporation, Madison, WI) to examine their bone mineral density (BMD).

Mechanical testing

Dissected left mandibles were stored in frozen in 0.145 M/L NaCl and thawed on the day of testing at room temperature. Hemimandibles were subjected to 3-point bending tests to obtain the maximum load using an AGS-10kNG materials testing machine (Shimadzu Corporation, Kyoto, Japan) at a displacement rate of 0.5 mm/s. The hemimandible was placed with the lingual side down. The central loading point was aligned at the midpoint. A piece of 5-mm-thick rubber foam was used to distribute the load more evenly over the irregular mandible surface. Load and displacement data were collected and analyzed by a personal computer.

Bone histology and histomorphometry

The right hemimandible, which was not tested mechanically, was cut, including 2 mm of neighboring normal bone. The specimens were decalcified in buffered 14.5% EDTA (pH 7.3) for 20 to 30 days, dehydrated, and paraffin embedded. Each block was cut in 5-μm-thick sections in the axial plane and stained with hematoxylin and eosin. Bone histomorphometric analysis was performed on 4 sections for each sample using National Institutes of Health (NIH) Image analysis. Eight fields were randomly selected from each section and measured twice with a 3-day interval by a single, unbiased examiner who was blinded to the experimental groups. Bone volume/total volume (BV/TV, %, ratio of bone volume to the total tissue volume of distracted region) was analyzed as a bone histomorphometric parameter.

Statistical analysis

All data were presented as the mean and standard error of the mean. Bone densitometry, mechanical testing, and histomorphometric results were statistically analyzed by 1-way analysis of variance (ANOVA) to identify differences between groups. If the results from the comparisons between groups were significant, least significant difference multiple comparisons tests were used to explore the differences. P less than .05 was considered statistically significance.

RESULTS

The hydrogel showed satisfactory syringeability (Fig. 2) and biocompatibility, with no sign of inflammation in injected areas. All 23 rabbits in the 4 groups tolerated the experimental procedure well, with weight loss of less than 10%. In all groups of the animals, lengthening of 8.61 ± 0.79 mm was successfully achieved and bone consolidation was obtained by the end of the experiment. No difference (P > .05) in regenerated bone dimensions was observed among the 4 groups (Table I). No nonunion was observed in all the specimens.

Radiographic examination and 3-point bending

Radiographs showed that there was more bone formation in group 1 than in any of the other 3 groups. BMD of the new bone in the distraction gap in group 1 was significantly greater (P < .05) than in the other 3 groups (Fig. 3). The maximum load in group 1, which received injections of Col/nHA/Alg hydrogel containing hNGFβ, was significantly higher (P < .05) when compared with groups 2, 3, and 4 (Fig. 4).
Bone histologic and histomorphometric analysis

At 2 weeks postlengthening, the distraction gaps of the animals were completely united with bone tissue. In group 1, distraction gaps mainly consisted of well-organized woven bone and lamellar bone, formed in parallel to the distraction forces, with signs of callus remodeling and no fibrous or cartilaginous tissues. In group 2, the bony trabeculae had various degrees of consolidation with rare fibrous and cartilaginous tissues and there was initial replacement of woven bone by lamellar bone. In groups 3 and 4, the distraction gaps had various degrees of consolidation, and all animals had cartilaginous tissues in the regenerates. In comparison, bone consolidation and remodeling were most advanced in group 1 (Fig. 5). At the 14-day consolidation, BV/TV in group 1 was significantly higher (P < .05) compared with groups 2, 3, and 4 (Fig. 6).

DISCUSSION

In clinical practice, injuries to nerves and the rather long duration of bone consolidation phase are 2 major problems in mandibular DO. Although many attempts have been tried to shorten the bone consolidation period during DO,15 such as low-intensity pulsed ultrasound, proper application of growth factors remains a viable strategy for this purpose.16,17 Nerve growth factors, which are vital in maintenance and regeneration of nerves,7 also proved to be able to stimulate differentiation and inhibit apoptosis of osteoblastic cells,9,18 and play an important role in bone regeneration. We have previously demonstrated that repeated injections of hNGF solution to the regenerate following the end of distraction could significantly increase myelinated fiber density of the IAN, as well as enhance bone consolidation in a rabbit model of mandibular DO.10,11 However, when the administration of hNGF was reduced to only once in the present study, very limited effect on bone formation was observed. This can be attributable to the fact that human NGF can be cleared from the body within comparatively short time periods because of enzymatic degradation. Although the exact degradation speed of the human NGF in vivo is unclear and further study is needed, the application of a delayed release system would be helpful. With the continued improvement of drug delivery method, NGF has been incorporated into some carriers to allow its slow release.19 Although the injectable hydrogel, as a drug delivery system, may elevate the efficiency of NGF application, they may rapidly release most of their protein content at an initial stage. Thus, to overcome this limitation, we incorporated a well-characterized collagen/nano-hydroxyapatite material into alginate hy-
drogel to fabricate a microparticle/hydrogel system. Data showed that the group receiving hNGFβ in Col/nHA/Alg hydrogel had superior histology of new bone than that of hNGFβ injection with saline. This indicated that hNGFβ was kept from its rapid degradation and was able to retain its biological activities for a prolonged period until its release from the microparticle/hydrogel system. The data also showed that the group receiving Col/nHA/Alg hydrogel alone had better new bone formation than the group receiving isotonic saline alone, which denoted the osteoconductive activity of Col/nHA particles, but it is not statistically significant.

The rabbit models of leg or mandible lengthening are well established and have been used extensively to perform callus stimulation studies. A relatively safe rate of bone lengthening was recommended to be about 1.0 mm/d in several animal models of mandibular DO, and rates faster than 2 mm/d could result in a poor quality of bone formation. In our present animal model, a demanding situation was deliberately produced to test the ability of intervention to bone consolidation.

In DO, tension-stress is believed to be the key stimulator of varied cells, in which expression of growth factors increases as long as the distraction is maintained and decreases when the distraction is completed. Several previous studies in animal models of mandibular DO have found that the expression of NGF and its receptors in the IAN peaked 1 week after the end of the distraction period, with the increased NGF levels persisting for approximately 2 to 4 weeks before beginning a gradual decline. Thus, the first week following the end of the distraction period is therefore likely to be a key period with insufficient endogenous NGF. Consequently, proper use of exogenous NGF may protect nerve fibers from degeneration and enhance nerve and bone regeneration.

In conclusion, we have demonstrated that a single injection of hNGFβ in Col/nHA/Alg hydrogel at the
end of the distraction period significantly enhanced new bone formation in a rabbit model of distraction osteogenesis, although future improvement of the evaluation should include the whole healing process until the end of optimal consolidation. It provides a possible way to shorten the consolidation phase of DO.

REFERENCES


Reprint requests:
De-lin Lei, DDS
Department of Oral and Maxillofacial Surgery
Fourth Military Medical University
School of Stomatology
Xi’an 710032, China
leidelin@fmmu.edu.cn