Curvilinear Distraction Osteogenesis with a New Internal Curved Distractor in Beagles

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Research

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Abstract

Background: In order to realize the curved distraction osteogenesis to treat complex jaw deformities, our team have invented and manufactured a new internal curved distractor to investigate the feasibility of using the new internal curved distractor in the application of mandible extension in dogs.

Methods: A mandibular osteotomy section was made in each of 6 adult dogs. The internal curved distractor was implanted. After 7 days of latency, the distraction was performed at the rate of 1mm per day, followed by 12 weeks consolidation period. CT examination is performed during the distraction process and the consolidation period. At the end of the consolidation period, sacrifice the dog and take specimens. The mandibles were evaluated by X-ray examination, general observation, Micro-CT and histological analysis.

Results: Curvilinear distraction was successfully performed in 4 of the 6 beagles. Compared to the left side of mandibles, the right sides were extended in both the length and height. Radiographically, there was no visible difference in the osteogenic density between the distraction gap and the natural bone. Micro-CT three-dimensional reconstruction found that the trabecular bone arrangement was directional and consistent with the direction of the distraction. Histological examination showed mature new bone, and large trabecular bone parallel to the direction of distraction was seen. The results further demonstrate the consistency of new bone osteogenesis direction and distraction direction.

Conclusion: These results suggest that by using the new internal curved distractor, mandibular curved distraction osteogenesis was achieved in beagles.

Background

Mandibular defects that arise from congenital mandibular hypoplasia, trauma, oncological resection can cause maxillofacial morphology abnormalities and dysfunction. Traditional repair methods such as autologous bone grafting is limited due to source scarcity, complications, and prolonged repair time. The special anatomical shape of mandibular also severely restricts the repair effect[1–3]. Since 1990, MaCarthy successfully used the distraction osteogenesis (DO) for the first time to achieve the extension of the mandible, solving the difficult problem of traditional surgical approaches[4]. In recent years, DO has been widely used in the treatment of maxillofacial surgery deformity and reconstruction of the jaw[5]. Due to the multiple curved structures of the jaw, the linear distractor cannot achieve the desired effect, and there are obvious limitations. The study of internal curved distraction osteogenesis has become one of the main research directions, however, the external distractor commonly used at present has some shortcomings, such as large volume, complex structure, easy collision and affecting the beauty and daily life. It is easy to cause local edema and infection, facial nerve injury, and scar left behind after distraction[6–8]. Therefore, the internal curved distractor is more in line with the real distraction requirements. With the development of various technologies, the development of built-in arc stretchers has gradually increased, but there is still a lack of curved distractors that can be popularized for clinical use.

In order to make new bones closer to the jaw contour to correct complex jaw deformities, Our research group developed a new internal curved distractor[9], which consists of a curved guide rail, a retainer plate, a connecting plate, a drive rod, a propulsion screw and a transport plate (Fig. 1). By rotating the cardan joint, the propulsion screw is driven to rotate, and the screw advances the transport plate to move along the curved rail, thereby achieving curved distraction osteogenesis. Thread pitch on screw is 1 mm, when distracting, rotate the drive rod 180° clockwise to rotate the propulsion screw 180°, the transport plate moves forward 0.5 mm. This study verified the feasibility of the distraction osteogenesis with this distractor by animal experiments.

Material And Methods

Six healthy adult male beagles aged 7–8 months, and weighting 9–11 kg were studied. Adaptive captivity for 1 week before the experiment. (The study was approved by the respective Ethics Committee of Medical ethics committee and the number of the protocol approval is KY2017031.)

15 minutes before the operation, beagles were given subcutaneous injection of atropine sulfate 0. 1 mg/kg, and then anaesthetized with an intramuscular injection of zoletil50 10 mg/kg. Pave sterile sheet after routine operation area disinfection. Injection of 2% lidocaine hydrochloride was given in the right surgical area of the animal (5 ml local anesthesia). Incision along the lower edge of the inferior border of right mandible, flap, expose the inferior border of mandible, and the curved distractor was placed and the installation position of the fixing plate and transport plate was confirmed. The electric motor drilled holes and the titanium screw was used to temporarily fix the distractor. Loosen the titanium screw and remove the distractor. The mandible was completely cut through along the osteotomy line with the electric saw blade. Then fix the curved distractor on both end sides of the mandible with titanium screws (Fig. 2). A 3 mm long incision was made from the distal skin of the incision to allow pass by the rod of the distractor. A large amount of saline rinse the wound and adequate hemostasis.

After a 6-day latent period, the curved distraction osteogenesis was started at a rate of 0. 5 mm twice a day continuously. By the end of the distraction period, the resistance to distraction gradually increases. The distraction period ends when the distraction rod could not be turned due to intense resistance. At the end of distraction period and end of consolidation period, the scan of head CT (iCAT 17–19, USA) was examined, under general anesthesia.

The beagles were euthanized under general aneasthesia after 12 weeks' consolidation, and the bilateral mandibles were harvested to observe the morphology of osteogenesis and measure the three-dimensional size. The X-ray examination was performed (50 mA, 60 kV, 50 s). New bone specimens in distraction osteogenetic gaps were divided into concave and convex parts. The cortical bone at the lower edge of the mandibular body and the cancellous bone of the condylar process were taken as nomal controls. After fixation for 1 week with 4% paraformaldehyde, the specimens were processed by micro-CT scanning (SkyScan 1172, Belgium). Three-dimensional images were made using CTvox software. At the center of each specimen, a plurality of $1.5 \times 1.5 \times 1.5$ (mm^3) cubic regions of interest were randomly taken for reconstruction and calculation. The system calculates a series of data: bone mineral density (BMD), bone volume fraction (BV/TV), trabecular thickness (Tb. Th), trabecular bone number (Tb. N), trabecular bone separation (Tb. Sp), etc.

The regenerated bone masses, normal cortical bone and condyle cancellous bone were cut off, decalcified, dehydrated, and embedded in paraffin. Sections 3 μm thick were cut longitudinally in the axial plane with a microtome, stained with HE and Masson staining for light microscopy.

The analysis was performed using SPSS16.0 statistical software, and the comparison between multiple samples was performed by one-way ANOVA. When the variances of the groups were homogeneous, the pairwise comparison between groups is performed by LSD-t test. If the variance was not homogeneous, the Tamhane'T2 method was used, and the difference was statistically significant at $P < 0.05$.

Results

One of the six beagles in the postoperative recovery process, the drive rod of the distractor broke due to the scratch and collision of the animal. Another animal had wound infection after operation, which could not be completely controlled by debridement and anti-infection therapy. The remaining 4 animals completed the curved distraction osteogenesis (10 days, 15 days, 18 days, 21 days respectively). After the end of the distraction period, the distractor was kept fixed for 12 weeks.

At the end of distraction stage, CT examination showed that the transport plate was pulled away from the osteotomy line along the curved guide rail, and the distraction gap gradually increased, fan-shaped distraction gap between the two bone sides (Fig. 3a). At the end of the consolidation period, CT examination showed that the distraction gap was filled by the new curved bone tissue, and the original osteotomy line was not visible (Fig. 3b). The X-ray findings of the specimen at the 12-week consolidation period: there was no distinguishable difference between the new bone in the distraction gap and the normal bone density. Compared to the Contralateral normal side of mandibles, the distraction sides were extended in both the length and height (Fig. 4).

The specimens after 12 weeks of consolidation period showed that mandibles were extended, the length and height of the mandible were separately increased by 15.5 ± 5.5 mm and 4.6 ± 1.6 mm. The new bone was curved and consistent with the curvature of the distractor. The boundary between the new bone and the original natural bone was indistinguishable, but the surface was slightly rougher than that of the normal bone (Fig. 5).

The new bone in distraction gaps has matured mature after 12 weeks' consolidation, and its trabecular bone density and thickness were not as much as that of the cortical bone in the mandibular body, but much higher than the condylar cancellous bone (Fig. 6). New bone specimens were divided into the concave part and the convex part. The analysis showed that the BMD (concave parts > convex parts), BV/TV (concave parts > convex parts) and Tb. Sp (concave parts < convex parts) values between the two segments were significantly different ($P < 0.05$), and there was no significant differences in Tb. Th and Tb. N values ($P > 0.05$). The osteogenesis quality of the concave parts of the new bone was higher than that of the convex parts (Fig. 7).

HE staining showed that the distraction zone was typical of mature bone at 12-weeks consolidation, and the distraction area showed reticulated thick trabecular bone and mature harvard system. The trabecular bones connect and fuse with each other,and the arrangement of the trabecular appeared to parallel with the curved distraction. Lamellar bones were arranged closely along the Harvard canal. There were abundant osteocytes and increased bone lacunae. At the same time, there were many osteoblasts surrounding the trabeculae. There was no obvious difference in the structure and composition between the mature new bone and the original bone. Masson staining showed that there was extensive red stained trabecular bone in distraction area, only a small amount of blue stained trabecular bone, which might be related to the maturity of new bone and bone resorption and reconstruction. The other tissue characteristics were consistent with the results of HE staining. Comparing the new bones from the concave parts and convex parts showed that the trabecula was thicker and denser in the concave parts than in the convex parts (Fig. 8).

Discussion

The general trend of curved distractor is from external to internal, from larger volume to small and light, the biocompatibility of materials is continuously improved, and the manufacturing process is more refined. Moreover, the device type is also constantly breaking, which is of great significance for the realization of curved distraction osteogenesis.

The external curved distractor has been unable to meet the actual clinical needs. In recent years, a variety of new internal curved distractors have appeared. However, all of them have their own shortcomings and have failed to promote their clinical application. In the early days of the study, some scholars used wire traction to drive the transport plate to move the transport bone along the curved guide rails to achieve the curve distraction of goat mandibular angle defect. However, when the resistance is too large, it may cause the wire to break[10]. Feng et al[11] also used a similar driving method to successfully repair a large bone defect in the anterior teeth area of the maxilla. However, this design also has the above problems, and the new bone is the new bone deviates 2–3 mm to the palatal side of the curved guide rail, which is not conducive to later implant repair. Seldin[12] and Niu[13] have respectively developed a type of distractor, both of which use nitinol wire to manufacture the center screw and make it rotate in a curved track to achieve curved distraction. However, this type of retractor can only be applied to curved traction with small curvature, and the strength of nitinol wire is limited. When the resistance is large or the curvature of distraction is large, the nitinol wire is easily broken. In addition, due to the increased resistance to distraction in the second half of distraction, a second operation is required to remove blocking tissue in the defect area. The curved distractor with guide groove and steel ball designed by The external curved distractor has been unable to meet the actual clinical needs. In recent years, a variety of new internal curved distractors have appeared. However, all of them have their own shortcomings and have failed to promote their clinical application. In the early days of the study, some scholars used wire traction to drive the transport plate to move the transport bone along the curved guide rails to achieve the curve distraction of goat mandibular angle defect. However, when the resistance is too large, it may cause the wire to break[10]. Feng et al[11] also used a similar driving method to successfully repair a large bone defect in the anterior teeth area of the maxilla. However, this design also has the above problems, and the new bone is the new bone deviates 2–3 mm to the palatal side of the curved guide rail, which is not conducive to later implant repair. Seldin[12] and Niu[13] have respectively developed a type of distractor, both of which use nitinol wire to manufacture the center screw and make it rotate in a curved track to achieve curved distraction. However, this type of retractor can only be applied to curved traction with small

curvature, and the strength of nitinol wire is limited. When the resistance is large or the curvature of distraction is large, the nitinol wire is easily broken. In addition, due to the increased resistance to distraction in the second half of distraction, a second operation is required to remove blocking tissue in the defect area. The curved distractor with guide groove and steel ball designed by Zhang et al[14] can move the steel balls in the guide groove along the curved guide groove, and then apply the driving force to the transport plate, so that the distractor can achieve distraction osteogenesis in any direction. However, the distractor can only move forward, which cannot be achieved for clinically special situations that require retreat. The automatic curve distractor designed by Magill et al[15,16] realizes the closed-loop control of the distraction process by using the micro high-pressure hydraulic system, position feedback and digital controller. The actuator uses potential energy storage device and controlled released energy to provide distraction force. The distraction can effectively increase the distance between the broken ends of bone to a certain extent to effectively induce new bone formation between bones. But the damage caused by animal or implant environment will lead to sensor failure, which will affect the distraction effect. Some curved distractors have been used in clinical practice, but they were not widely used in clinical practice due to some deficiencies. The individualized three-focus curved distractor developed by Cai et al[17] has a trajectory designed according to the osteotomy of the mandible. However, after the distraction period, the new bone is still close to a straight line, and the shape is slightly smaller than the original mandible, which is inconsistent with the maxillary dental arch in the sagittal direction, and the patient needs to undergo an additional operation. Baek et al[18] designed a passive self-ligating bracket combined with a trifocal distraction-compression bone plate to reconstruct a large bony defect and multiple missing teeth. However, the stability of the thickness and vertical height of the regenerated alveolar bone and the success rate of the subsequent alveolar bone implant should be evaluated. Kaban[19] reported that the curved distractor developed by Synthes CMF Company uses the classic mechanical method of turbine drive to drive the bone to realize the curved distraction. However, the traction length of this curved distractor is limited, and it cannot be applied to large-scale curved defect areas, and the force rod gradually advances into the body with the distraction process, so a long enough force rod is needed. It causes inconvenience of operation, and there is a risk of bringing infection into the body. It can be seen from the above that the design of the existing curved distractors is not ideal, and an internal distractor with a more reasonable structure and better performance needs to be developed to meet the needs of mandibular curved defect repair and reconstruction. The new internal curved distractor developed by our group can achieve the sufficient strength for the operation, and the propulsion screw can move freely along the curved guide rail, the new bone is basically the same as the curvature of the distractor rail. At the end of the fixed period, the distractor was well fixed, and no loosening, fracture or deformation occurred, and the osteogenesis effect was also ideal. In addition, the curvature of the curved guide rail can be customized according to the distance of traction and the curvature size of movement required by the curved distraction.

The results of radiological examination, micro-CT analysis and histological observation of new bone showed that the bone mineral density of the concave parts is obviously higher than that of the convex parts. This is in accordance with the previous research results of Zhou, in which the difference of new bone formation in the distraction gap was attributed to the difference of distraction rate: since the trajectory of the transport plate is a curve, the distraction rate of the concave side (< 1 mm/d) was lower than the convex side (> 1 mm/d) [20].

There are still some shortcomings in this experiment: (1) In this study, beagles were selected as experimental animals. During the research, after installing the internal curved distractor in the beagles, due to the pain and discomfort after the operation, there were still uncontrollable external forces such as scratching and bumping that cause the damage of the force device that protrudes outside the body. Through the biomechanical analysis of three-dimensional finite element, this study verified that in the human body application process, under the mechanical condition of simulating the maximum bite force of human, In the initial, middle and final stages of the curved distraction movement, the stress distribution of the curved distractor is within the safe range. And the maximum stress on the mandible is located in the neck of the condyle (biomechanical analysis results will be reported separately). We assume that in the clinical application process, the distractor can be well and fully protected by dressing covering and bandage wrapping. At the same time, the patient's scratch, collision and other unexpected movements are controllable, so that the accidental breakage of the drive rod can be avoided during the animal experiment. (2) During the distraction period, as the bone transport plate advances, the angle between the propulsion screw and the guide rail changes and the ingrowth of surrounding soft tissue may cause clamping of the tissue and affect the distraction process. The team will further optimize the design of the internal curved distractor to avoid this problem.

Conclusion

In summary, through the research of this subject, it can be considered that: (1) The new internal curved distractor developed by us can realize the curved distraction osteogenesis. This new internal curved distractor is easy to operate and practical. It can be used for curved distraction osteogenesis of mandible. It can be used for correcting curved jaw deformity and repair curved bone defect of jaw. It provides a good new method and equipment for curved distraction osteogenesis. (2) The new internal curved distractor can be further advanced to the clinical trial stage. In the course of clinical trials, it is necessary to further observe the mechanical complications, infection risk of curved distractor; and further study the effect of distraction osteogenesis with different distraction rates and frequencies.

Declarations

Ethics approval and consent to participate

After review by the Medical Ethics Committee of the Stomatology Hospital of Guangzhou Medical University, the research�Curvilinear distraction osteogenesis with a new internal curved distractor in beagles�content and methods conform to the medical ethics norms and requirements.

reference number: KY2017031

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yang Zhou are joint first authors. Yang Zhou, Libin Zhou and Zhengguo Piao contributed to the conception, design, data acquisition and interpretation, and drafted and critically revised the manuscript; Zhicong Li, Haiqiong Yue contributed to the data interpretation, and drafted and critically revised the manuscript; Mi Yang, Qichen Liao, Fengpiao Chen and Xin Li contributed to the data acquisition, drafted and critically revised the manuscript.All authors gave final approval and agree to be accountable for all aspects of the work.

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References

1. Emerick KS, Teknos TN. State-of-the-art mandible reconstruction using revascularized free-tissue transfer. Expert Rev Anticancer Ther. 2007; 7:1781-1788. https://dx.doi.org/10.1586/14737140.7.12.1781
2. Li J, Ying B, Hu J, Zhu S, Braun TW. Reconstruction of mandibular symphyseal defects by trifocal distraction osteogenesis: an experimental study in Rhesus. Int J Oral Maxillofac Surg. 2006; 35:159-164. https://dx.doi.org/10.1016/j.ijom.2005.07.007
3. Sacco AG, Chepeha DB. Current status of transport-disc-distraction osteogenesis for mandibular reconstruction. Lancet Oncol. 2007; 8:323-330. https://dx.doi.org/10.1016/S1470-2045(07)70102-X
4. McCarthy JG, Hopper RA. Distraction osteogenesis of zygomatic bone grafts in a patient with treacher Collins syndrome: a case report. J Craniofac Surg. 2002; 13:279-286. https://dx.doi.org/10.1097/00001665-200203000-00016
5. Karp NS, Mc Carhty JG, Schreiber JS, Sissons HA, Thorne CH. Membranous bones lengthening: A serial histological study. Ann Plast Surg. 1992; 29:2. https://dx.doi.org/10.1097/00000637-199207000-00002
6. Ortakoglu K, Karacay S, Sencimen M, Akin E, Ozyigit AH, Bengi O. Distraction osteogenesis in a severe mandibular deficiency. Head Face Med. 2007; 3:700-707. https://dx.doi.org/10.1186/1746-160X-3-7
7. Annino DJ Jr, Goguen LA, Karmody CS. Distraction osteogenesis for reconstruction of mandibular symphyseal defects. Arch Otolaryngol Head Neck Surg. 1994; 120:911-916. https://dx.doi.org/10.1001/archotol.1994.01880330005002
8. Schendel SA. Curvilinear distraction. J Oral Maxillofac Surg. 2009; 67:2696-2697; author reply 2697-8. https://dx.doi.org/10.1016/j.joms.2009.06.026
9. The 306th Hospital of the Chinese People's Liberation Army. An internal curved distractor: China, CN201510051701. 2[P]. 2015-05-06. {URL}: http://www. wanfangdata. com. cn/details/detail. do?_type=patent&id=CN201510051701. 2
10. Zhou LB, Liu YP, Zhao JL, Wu ZY, Hu M, Chen BL, Sekou S, Li DC. Experimental study on repair of mandibular angle defect with internal curved distractor. Journal of Clinical Stomatology. 2005; 21:16-18. https://dx.doi.org/10.3969/j.issn.1003-1634.2005.11.006
11. Feng ZH. An experimental study of alveolar bone distraction osteogenesis to repair unilateral maxillary defect. Fourth Military Medical University. 2009. https://dx.doi.org/10.7666/d.d218788
12. Seldin EB, Troulis MJ, Kaban LB. Evaluation of a semiburied fixed-trajectory curvilinear distraction device in an animal model. J Oral Maxillofac Surg. 1999; 57:1442-4. https://dx.doi.org/10.1016/s0278-2391(99)90729-2
13. Niu XG, Han XX, Hao Y, Liu RF, Li GH. Clinical application of internal curved distraction osteogenesis for mandibular defects. Journal of Oral Materials and Devices. 2007; 16:213-216. https://dx.doi.org/10.7666/d.d218788
14. Zhang GH, Shang HT, Guo YX, Liu XY, He LS. A preliminary experimental study of curve distraction osteogenesis. Journal of Practical Stomatology. 2015; 31:153-156. https://dx.doi.org/10.3969/j.issn.1001-3733.2015.02.01
15. Magill JC, Byl MF, Goldwaser B, Papadaki M, Kromann R, Yates B, Morency JR, Kaban LB, Troulis MJ. Automating skeletal expansion: an implant for distraction osteogenesis of the mandible. J Med Device. 2009; 3:14502. https://dx.doi.org/10.1115/1.3071969
16. Goldwaser BR, Magill J, Papadaki ME, Byl M, Kromann R, Yates B, Morency J, Kaban LB, Troulis MJ. Continuous mandibular distraction osteogenesis: novel device and preliminary results in minipigs. J Oral Maxillofac Surg. 2013; 71:e168-e177. https://dx.doi.org/10.1016/j.joms.2012.09.012
17. Cai M, Lu XF, Shen GF, Cheng HJ. Individualized three-focus arc retractor for repairing mandibular large Type defect. Chinese Journal of Oral and Maxillofacial Surgery. 2013; 11: 113-118. {URL}: http://kns.cnki.net//KXReader/Detail?TIMESTAMP=637078994798382500&DBCODE=CJFQ&TABLEName=CJFD2013&FileName=ZGKQ201302004&RESULT=1&SIGN=0DcMzau%2fKrzqMRpy

18. Baek SH, Kim NY, Paeng JY, Kim MJ. Trifocal distraction-compression osteosynthesis in conjunction with passive self-ligating brackets for the reconstruction of a large bony defect and multiple missing teeth. Am J of Orthod Dentofacial Orthop. 2008; 133: 601-611. https://doi. org/10. 1016/j. ajodo. 2006. 04. 037
19. Kaban LB, Seldin EB, Kikinis R, Yeshwant K, Padwa BL, Troulis MJ. Clinical application of curvilinear distraction osteogenesis for correction of mandibular deformities. J Oral Maxillofac Surg. 2009; 67: 996-1008 https://dx.doi.org/10.1016/j.joms.2009.01.010
20. Zhou L, Shang H, Feng Z, Liu C, Ye W, Ma Q, Wu W, Liu Y. Segmental curvilinear distraction osteogenesis. Med Hypotheses. 2012; 79: 427-429. https://dx.doi.org/10.1016/j.mehy.2012.06.011

Figures

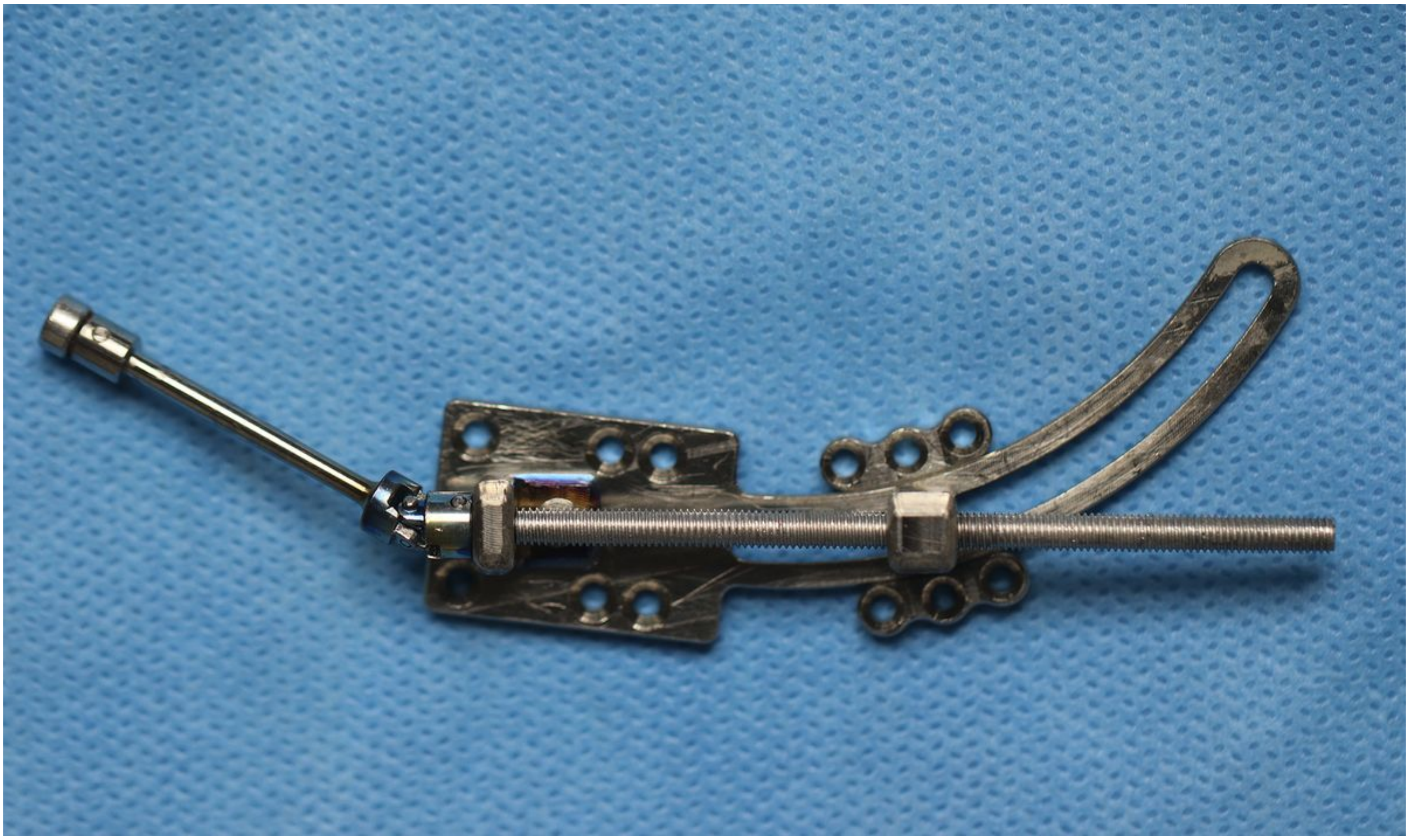

Figure 1

new internal curved distractor (Thread pitch on screw is 1 mm, when distracting, Rotate the drive rod 180° clockwise to rotate the propulsion screw 180°, the transport plate moves forward 0.5 mm.)

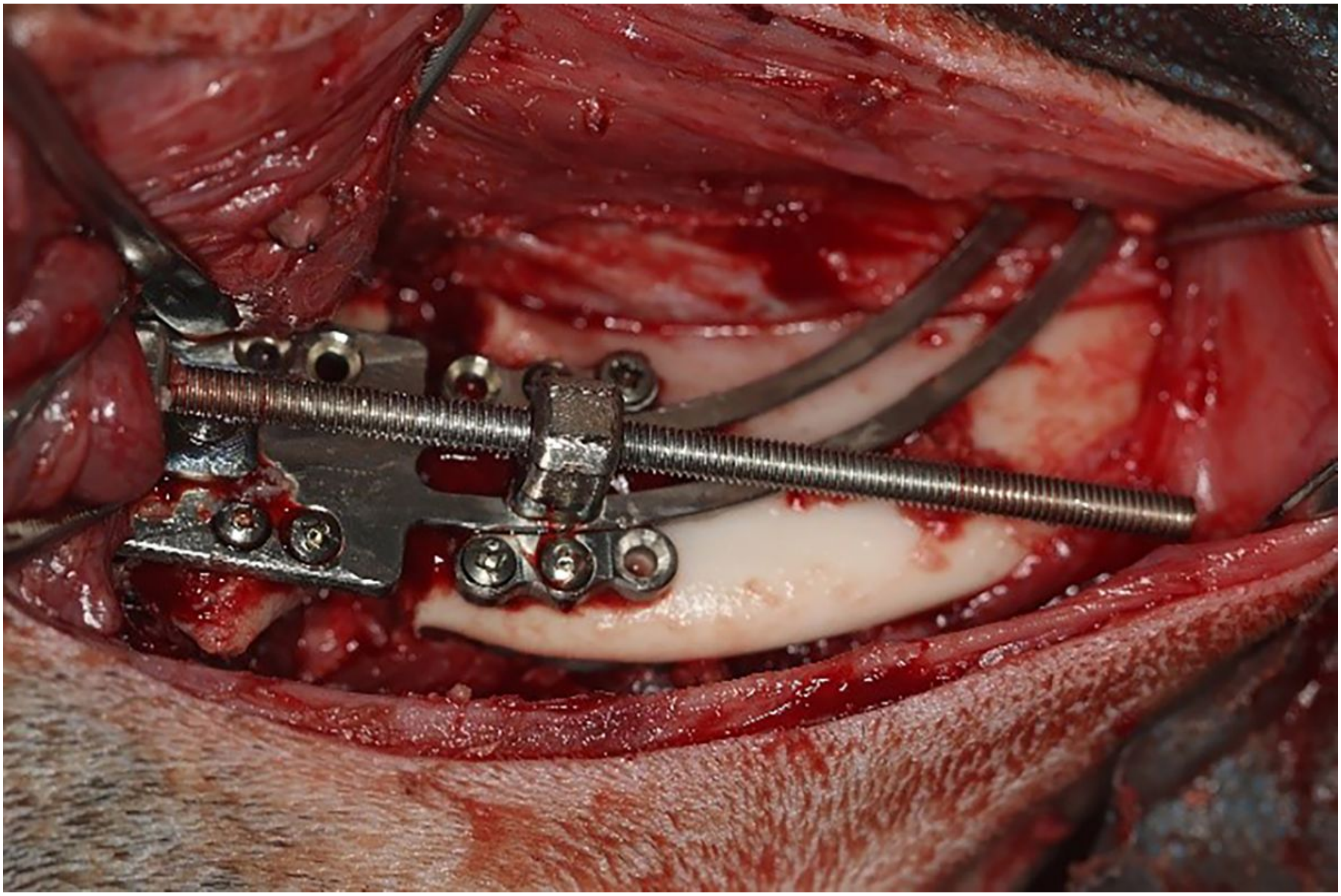

Figure 2

Osteotomy and distractor placement

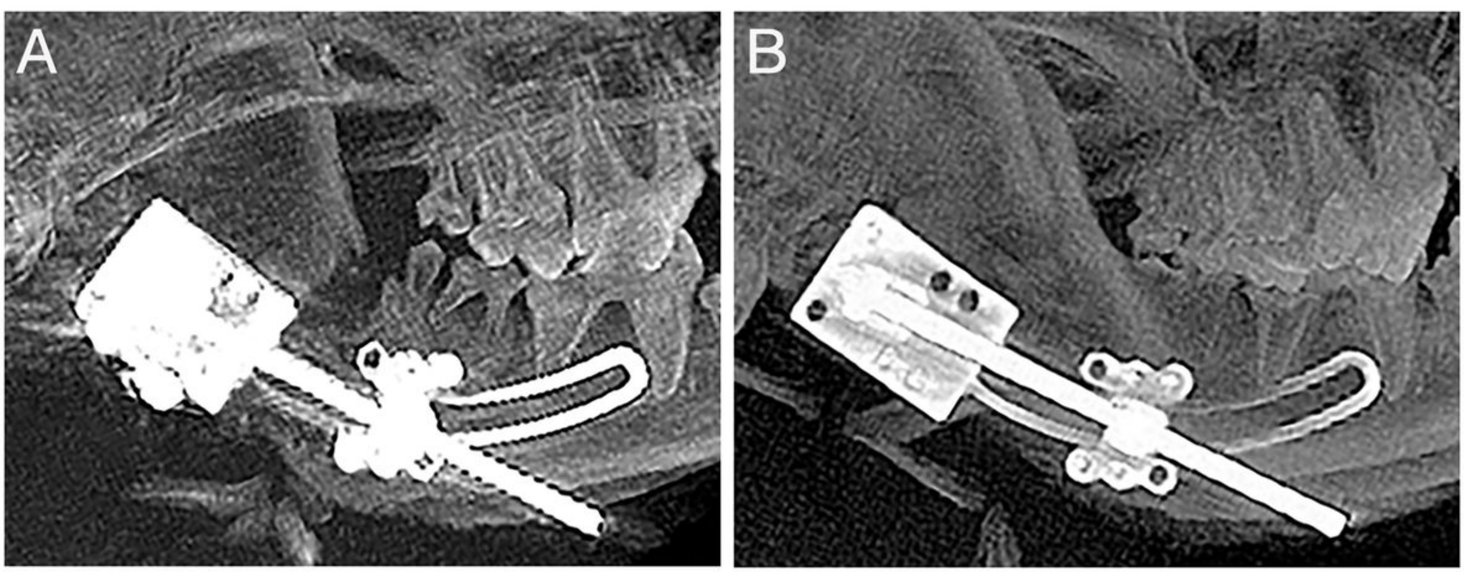

Figure 3

CT images of experimental side during distraction:（A）CT image at the end of distraction showed sector-shaped bone distraction gap;（B）CT image at the end of consolidation period showed bone healing within distraction gap.

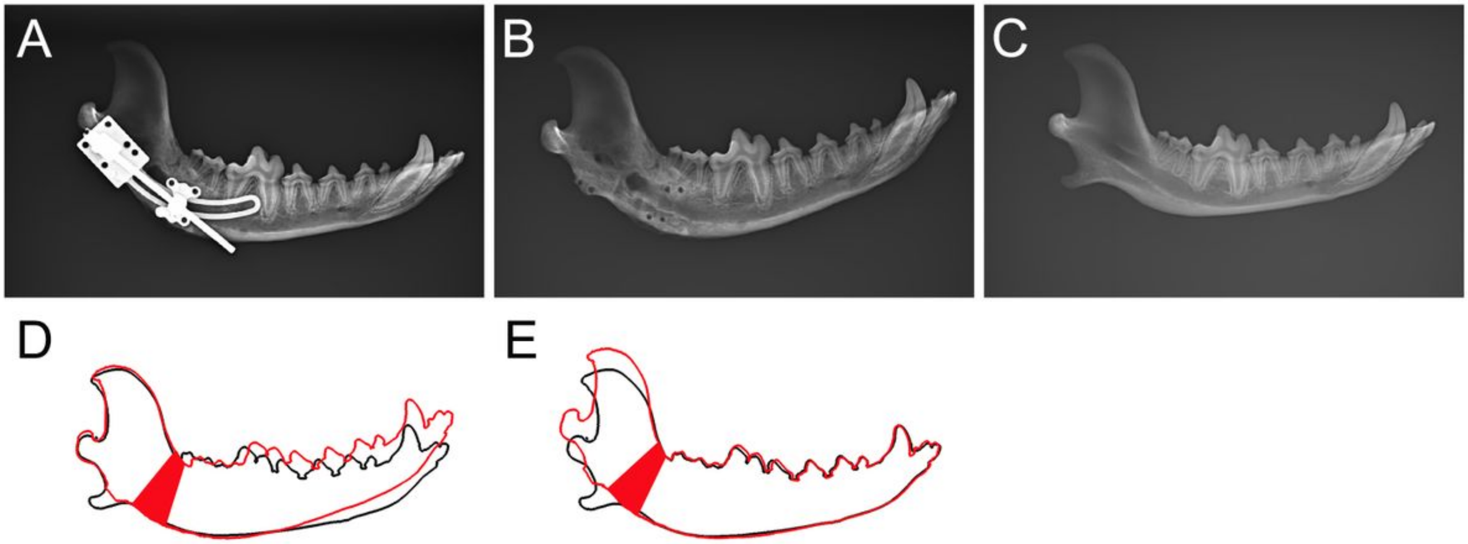

Figure 4

The X-ray findings of the specimen at the 12-week consolidation period: (A) distraction side (with distractor); (B) distraction side (without distractor); (C) Contralateral normal mandible; (D) fusion of the mandibular ramus of the two sides showed the body of the mandible was extented forward and upward; (E) fusion of the mandibular body of the two sides showed the ramus was extented backward and upward.

Figure 5

The gross specimen after consolidation period(A) distraction side (with distractor); (B) occlusal viewarrow indicate the distraction side); (C) buccal view; (D) lingual view.

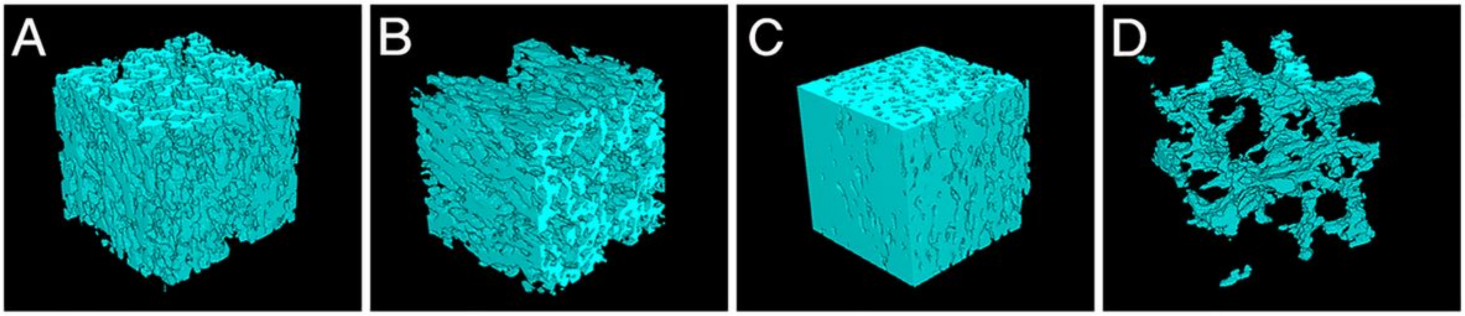

Figure 6

region of interest of specimens: (A) new bone formation of the concave part;Bnew bone formation of the convex part; (C) normal cortical bone; (D) condylar cancellous bone.

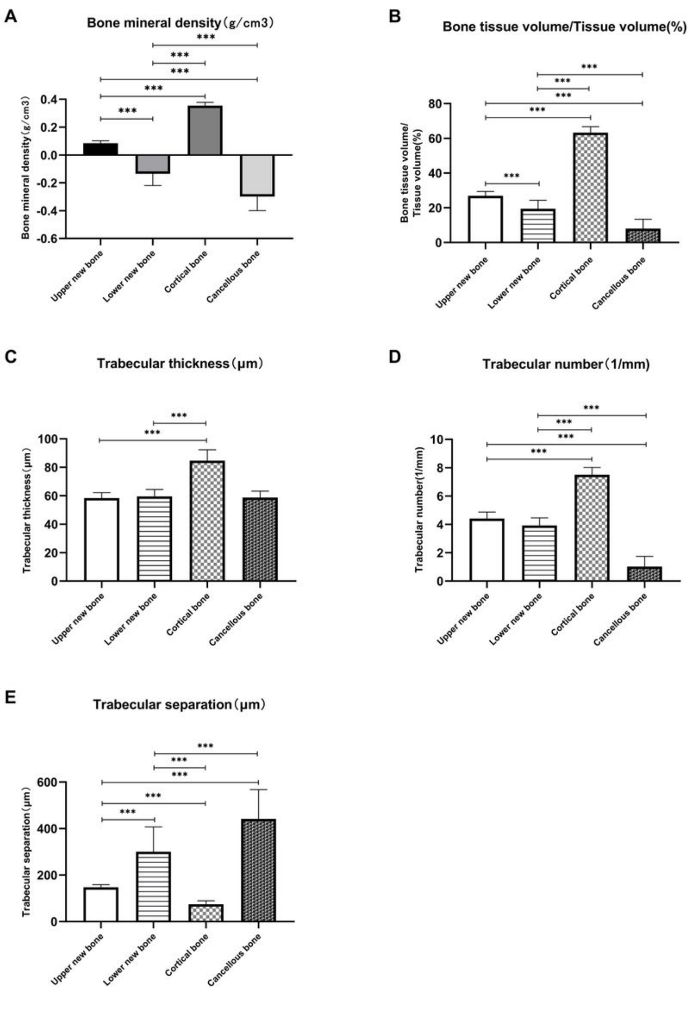

Figure 7

Statistical analysis of BMD, BV/TV, Tb.Th, Tb.N and Tb.Sp in each group after 12-weeks' consolidation. * P<0.05, **P<0.01, ***P<0.01

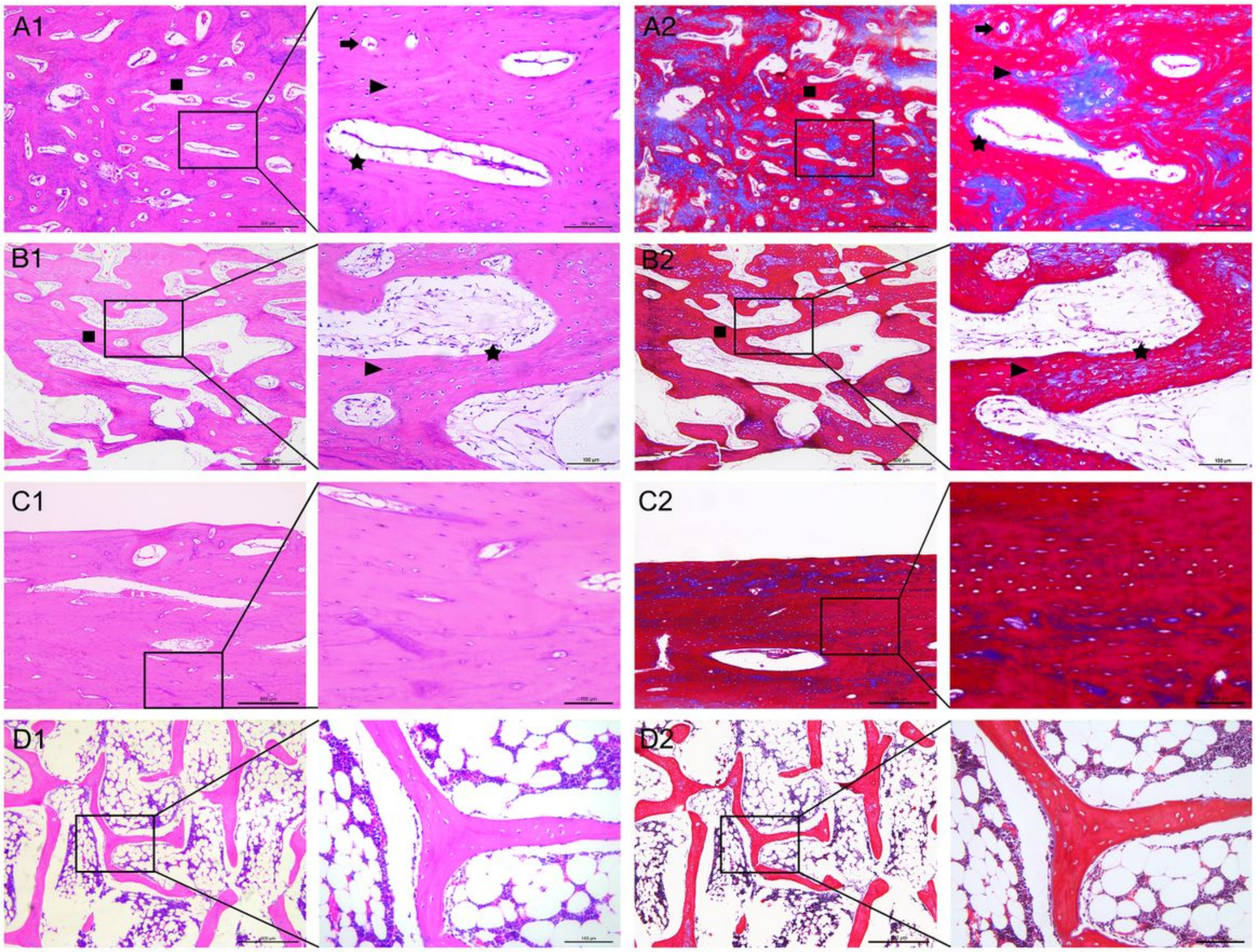

Figure 8

New bone formation of the concave part (A1, A2): the trabecular bone gradually loses its directionality; new bone formation of the convex part (B1, B2): trabecular bones parallel and regular arrangement along the direction of distraction; cortical bone (C1, C2); cancellous bone (D1, D2). (A1, B1, C1 and D1) HE staining; (A2, B2, C2 and D2) Masson staining. Original magnification: 50 × and 200 ×. Square: trabecular bone; triangle: osteocytes and bone lacuna; arrow: Haversian canal; pentagram: osteoblasts