Published in final edited form as:
J Pediatr Orthop. 2010 June ; 30(4): 365–370. doi:10.1097/BPO.0b013e3181d8fa74.

Vertebral Growth Modulation by Electrical Current in an Animal Model: Potential Treatment for Scoliosis

George R. Dodge, Ph.D.[*], **J. Richard Bowen, M.D.**, and **Changhoon Jeong, M.D.**
Bone & Cartilage Research Laboratory, Nemours Biomedical Research, & Dept. of Orthopaedics, Alfred I. duPont Hospital for Children, Wilmington, DE 19899; University of Delaware, Deparment of Biological Sciences, Newark DE 19716

Abstract

Background—The concept of modulating spinal growth to correct scoliosis is intriguing, and this study proposes a new model. Inhibition of vertebral growth on the convex side of a curve would allow continued normal growth on the concave side to correct the scoliosis. In a previous study, we induced bony bridges across the physis of the femur producing an epiphysiodesis in rabbits by using a stimulator modified to deliver a current of 50 μA. The present study builds on this finding to design a model with an aim of inhibiting growth in a unilateral peripheral portion of the vertebral endplate physis, which induces asymmetric spinal growth.

Methods—The study was conducted with 8-week-old rabbits; six were treated with electrical current via an implantable 4-lead device; three were age matched normal rabbits. The device was implanted and delivered a constant current of 50 μA from each electrode, continuously for 6 weeks. Weekly radiograph monitoring and endpoint histology were performed.

Results—Spinal growth was modified by inducing asymmetric growth of the vertebra of young rabbits using electric stimulators delivering 50 μA of direct current through electrodes implanted in a left peripheral portion of the endplate physis.

Conclusion—This concept study, based on our previous study, involved a method and device for inhibiting growth in one aspect of the vertebral endplate using electrical current at an amplitude that induced a hemiepiphysiodesis. Our results demonstrated that this technique both establishes an in vivo model of scoliosis and suggests that if this technique were applied to an existing curve it could potentially induce asymmetrical growth of the spine, thereby correcting scoliosis by continuing the normal growth on the concavity of the curve.

Clinical Relevance—A potential new method for modulating spinal growth was developed, and, with further research, this method may be useful in treating children with scoliosis by delivering a growth-inhibiting current to the physeal areas of vertebra through electrodes placed percutaneously.

Keywords

model of scoliosis; spinal growth modulation; electrical stimulation; vertebral epiphysiodesis; vertebral hemiepiphysiodesis

[*]Corresponding author (current address): George R. Dodge, Ph.D., McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, 424 Stemmler Hall, 36th Street and Hamilton Walk, Philadelphia, PA 19104-6081, Phone: 215-573-1514; Fax: 215-573-2133; gdodge@upenn.edu.

Introduction

Scoliosis is a lateral curvature of the spine and is a common condition in children that may progress to severe curves. Currently, orthoses are used often in children with progressive curves between 25 to 40 degrees with the aim of inhibiting curve progression; however, orthotic efficacy is questionable, and some curves progress in spite of the bracing. In severe curves with magnitudes over about 50 degrees, spinal fusion may be used to reduce the magnitude and stabilize the curves. Both orthoses and spinal fusion have many unappealing aspects, and, therefore, an alternative method is needed for correcting the curvature of the spine in children with scoliosis.

Modulation of spinal growth is a possible alternative treatment method in which spinal growth is altered to allow curve correction. Modulation of spinal growth involves limiting the anterolateral growth of the vertebrae at the apex of a curve so that remaining contralateral growth in the concavity corrects the scoliosis. Treatment strategies for spinal modulation include using staples, tethers, or mechanical disruption of vertebral growth.[1-11] These techniques require operative procedures with some inherent risk, which led us to consider alternative methods for consistent and reliable modulation of spinal growth.

There are case examples of children with localized physeal growth inhibition from noxious energy sources such as radiation, frostbite, or freezing. Radiation therapy has been used in children to treat malignancies near the spine such as in Wilms tumor and neuroblastoma. When the therapeutic radiation was applied asymmetrically to the spine in young children, scoliosis developed as the vertebral bodies became wedge shaped from peripheral-lateral growth inhibition.[12-14] Radiation therapy is obviously too risky to consider as a treatment method for scoliosis. Also, frostbite of the extremities in children sometimes causes asymmetric growth inhibition resulting in angular deformities. Repetitive freezing of the vertebral endplate physis at a lateral/peripheral area in rabbits was tested in our laboratory; however, the degree of modulation of spinal growth was inconsistent. Recently in our laboratory, a current of 50 μA was shown to produce consistently reliable growth inhibition compared with 20 μA, and at the higher current there was an induction of bone bridges in the physis of rabbit femurs.[15] The technology uses a power source and one electrode or a series of electrodes for applying a current sufficient to reduce or stop the physeal growth of a bone. Asymmetric physeal growth inhibition developed when the current was applied through electrodes that were placed in only a peripheral region of the physis.

The aim of this study was to determine if asymmetric growth inhibition of the vertebral endplate could be generated with electrical current in an animal establishing a new model for spinal growth modulation. This study is one of the sequential steps in developing a treatment to correct progressive curves in children with mild adolescent idiopathic scoliosis by placing percutaneous electrodes that deliver a very small current into selected areas of the vertebral endplate to produce controlled growth modulation in the spine.

Materials and Methods

Animal model and device

The study was conducted adhering to an Institutional Animal Care and Use Committee-approved protocol. This study was designed to implant an electrode into one half of the growth plate of the rabbit spine with the outcome being to unilaterally affect the growth of the vertebral body, hence creating a model of scoliosis. The study was conducted using 8-week-old (skeletally immature) female New Zealand white rabbits; six were treated with electrical current via an implantable 4-lead device and three rabbits were age matched normal animals. The rabbits are considered as “adolescence” and in an active growth phase for the duration of

the study. In this study these were chosen as control over animals with lower or no current since this had been done in a previous study showing no marked effect on growth. [15] The 4-lead device was implanted in the 6 immature animals delivering a constant of 50 μA from each electrode, and the current was delivered continuously for 6 weeks.

The constant current delivery system consisted of an implantable generator, which housed the battery and electronics and served as the anode, connected via an insulated lead to a thin titanium cathode. It was similar to the electrical bone growth stimulator that is currently available for clinical use (Osteogen, EBI, Parsippany, NJ). The electrical stimulator delivered a constant current of 50 μA (2.2 -2.4 volts) during the 6 weeks. The amplitude of delivered current was confirmed by a specially designed tester that monitored a telemetry signal from the stimulator proportional to the in vivo current. The electrical stimulator was tested at the time of implantation and was reconfirmed when the animals were euthanized to ensure accuracy of the delivered current.

Surgical technique and postoperative care

After a pre-anesthetic injection of xylazine (4 mg/kg IM) followed 10 minutes later with ketamine (50 mg/kg IM), the electrical stimulation device was implanted. The implantation of the electrodes was performed with the animals under anesthesia, and surgery was typically completed within 40 minutes. With the use of aseptic technique, an oblique skin incision was made on the left side of the back from just below the 12th rib to the L4 vertebral level. The back muscles were dissected back and retracted laterally and one electrode was inserted into the caudad physeal endplate of the L1, two in L2 (cephlad and caudal), and one in the L3 cephlad endplate (for a total of four electrodes). The electrodes were inserted in the following manner: under image intensifier control, a fine needle (24G) was directed from laterally to medially for a depth of 10 mm into the left aspect of 4 vertebral endplates, and the needle was withdrawn to create a “needle tract” hole. Then, a fine wire electrode was placed tightly into the “needle tract” hole of the vertebral bodies. Radiography confirmed the position of the electrodes during and at the end of the surgical procedure. The electrode leads and power source were sutured in place in adjacent subcutaneous tissue, and the wound was closed with absorbable sutures and dressed with sterile iodine. After surgery, the rabbits were housed in an approved animal facility, where all animals were given identical diet and water supplies. On the 6th week, the animals were euthanized using a commercial veterinary euthanasia product (Sleepaway®, 0.4 ml/lb IM; Ft. Dodge Laboratories, Inc., Ft. Dodge, Iowa).

The electrical stimulation packs were modified Osteogen devices typically used for enhancing spine fusions (provided by EBI, L.P., Parsippany, NJ). The modification consisted of increasing the current delivered to each electrode to 50 μA.

Radiographic assessments

Standard spine anteroposterior (AP), oblique, and lateral radiographs were taken every week after the operation for the evaluation of the process of hemiepiphysiodesis. Radiographs were taken while rabbits were under anesthesia, held only by their front legs permitting free movement of their trunk and lower extremities. The three normal age matched rabbits who did not receive surgery were also analyzed. Measurements of vertebral height of both sides of the vertebral body from D12 to L4 and Cobb angle between L1 and L3 were made on AP radiographs. By 6 weeks after surgery, final anterior/posterior radiographs of the spine were made. The animal was euthanized, and the spines were removed and prepared for decalcification and H&E histology. After euthanizing the animals, the spines were extracted and cleared of ligaments or muscles. Radiographs were taken before and after dissection.

Histologic analysis

All specimens were fixed in formalin (10% neutral buffered formalin), and decalcification was performed with ethylenediaminetetraacetic acid (EDTA) using the following solution: 37.22 g of EDTA was dissolved in 1 L of distilled water, then 70 ml of a concentrate of hydrochloric acid (HCl) was added. Histological sections of 4 micron in an anterior to posterior sagittal direction of the vertebral segments were stained with H&E, Alcian blue, and Safranin-O (shown).

To reduce the variance in histological sections, similar regions of the growth plate from both the medial and lateral undulated ridges were evaluated. We studied the narrowing of the growth plate and its possible closure, bony bridges, and the regular or irregular cellular arrangement of various layers in the growth plate.

Results

The purpose of this study was to test if the growth of the spine could be altered in a unilateral manner by placing electrodes in four epiphyseal plates of three vertebral bodies (one in L1, two L2, one in L3). In the animals with the electrical current, there was consistent evidence from the radiographs that the growth of the spine was affected by the unilateral treatment. After the 50 μA current in four vertebral endplates as early as 3 weeks, it was obvious that the spine was curving toward the side where the current was delivered (concave on current side). In the three age matched normal rabbits, the spine remained straight during the 6-week period (Fig. 1). The radiographs were digitized, and the height of the vertebral bodies was measured (Table 1). The differences between right and left were determined and mean percent of height discrepancy was determined for each group. The discrepancy of both side vertebral heights in the operated vertebrae (L1, L2, L3) was significantly more than the discrepancy in the non-operated vertebrae (D12, L4). Given the data represents the mean of the group and the variation is apparent in the groups and regardless of if there was one or two electrodes. Even though the sample size was small, some measurements were significant ($p \leq 0.05$), and, in total, the data showed that the growth was consistently less in the operated side in all animals. The mean angular deformity measured by Cobb angle between L1 to L3 at 6 weeks was 8.4 ± 1.42 degrees (ranged from 6.9 to10 degrees), which was significantly different from the age matched normal rabbits ($p \leq 0.05$) (Table 2). By histology, it is clear that the treated growth plate is markedly more narrow with less of the typical organization (Fig. 2). More hypertrophic-appearing cells are present. The H&E stained sections shown are representative micrographs of the control and treated growth plate after 6 weeks with 50 μA. There is a noticeable decrease in the height of the growth plate as is evident in the micrographs (Fig 2). At the 50 mA it is quite clear that the current induced a reduction of endplate height in the region of the electrode and the image also shows a high degree of cellular disorganization and cell clustering or cloning. Note the images of low and high power are of the same magnification for normal rabbit vertebra and for that rabbit treated with for 50 μA.

Discussion

Severe scoliosis in children generally requires a spinal fusion to correct and control progression of the curves. Recently, fusionless treatment methods are being attempted with the aims of correcting or controlling the scoliosis. Dickson proposed that the etiology and progression of idiopathic scoliosis involved an imbalance of sagittal alignment.[16,17] Thoracic hypokyphosis or asymmetric anterior spinal overgrowth results in spinal rotation, which creates the three-dimensional deformity in scoliosis.[18,19] Modulation of spinal growth involves limiting the anterolateral growth of the vertebrae or disc at the apex of a curve so that remaining contralateral growth in the concavity corrects the scoliosis. Currently, treatment strategies for spinal modulation that restrict anterior spinal growth include the use of staples, tethers, or

mechanical disruption of vertebral growth.[1-11] In our laboratory, we have been exploring new approaches and methods for spinal growth modulation, which could be applied percutaneously and would cause controlled physeal growth inhibition of the vertebral body with resultant wedging and yet preserve disc function.

There are case examples of children with localized physeal growth inhibition from noxious energy sources such as radiation, frostbite, or freezing.[12-14] The effect of electrical current on formation of bone has been studied extensively; however, there are little data concerning the effect of current on inhibition of cartilage growth. Direct current (DC) bone growth stimulators have been used for at least 30 years to stimulate bone formation in spine fusions, fractures, and pseudarthrosis.[20-22] Direct current stimulators enhance bone formation in the vicinity of the electronegative cathode, which is placed in a bone graft to induce a fusion mass, at the fracture site to enhance callus, or in a nonunion to stimulate healing.23 The optimal current range for DC stimulators to induce new bone formation is 10 to 50 μA depending on cathode length and material and anatomical placement site.[24-26] There has been no reported change in the growth plate of children when DC electrical current of 20 μA is applied for the purposes of fracture healing or bone fusion.[27-29] Various studies have shown different effects on the electrical stimulation of the physis (stimulation of growth).[26,30] Interestingly, one potential mechanism for the effect of direct current on bone is the up-regulation of osteoinductive growth factors.[31] In our laboratory, we demonstrated in a previous study that a current of 50 μA, but not the clinically used 20 μA or a control device without current., would cause an inhibition of growth, i.e., an epiphysiodesis of the distal femoral physis in rabbits,15 and, as shown in the present study, a hemiepiphysiodesis can be induced by applying the electrical current asymmetrically in the distal femoral physis of rabbits. To be translationally relevant as possible the rationale for using the electrode device included the fact that titanium electrodes pass higher currents than do stainless steel without causing tissue necrosis, and that this cathode material and similar device (20 μA) is currently a FDA-approved implantable device. We built on our previous experiments demonstrating use of a higher current (higher than that which promotes bone formation) and explored the effect of 50 μA direct current to one side of the vertebral body. Evaluation was made over 6 weeks, and the results were a unilateral alteration in the longitudinal rate of growth. The inhibition of growth in the area of the endplate with the electrode is apparent in a reduction of vertebral body height.

While the study demonstrated the effect expected, further confirmation of our findings needs to come from elucidation of the biological mechanisms in which this level of electrical current modulates spinal growth. We did achieve vertebral growth modulation in the rabbits, and we did not observe disc changes. Using this model, our study supports our premise that electrical current can affect the growth of the vertebral endplates and result in a curvature. The study supports our hypothesis that this method could be used to limit growth in the convexity of an existing curve thereby limiting the progression of the curve and potentially correct the scoliosis in growing individuals. A test of this in a child-relevant sized animal model is under way.

In summary, this concept study involved a method and device for inhibiting growth in the distal femoral physis of rabbits by using electrical current at an amplitude that induced epiphysiodesis or hemiepiphysiodesis. Since the technology uses a power source and one electrode or a series of electrodes for applying a current sufficient to reduce or stop the growth of a bone to selected regions of the bone, it has the potential to be delivered percutaneously in appropriate spinal locations. This modulation if applied to vertebral growth would result in controlled wedging of the vertebral body on the convexity a curve to correct scoliosis without limiting disc mobility.

Acknowledgments

The authors thank Dr. Bruce Simon for expert advice regarding the electrical stimulators and Theresa Michel and Minwook Kim for help in preparing the manuscript. The study was sponsored in part by the grants from the NIH,AR045242, EBI/Biomet, Inc., and Nemours.

Financial support provided by EBI-Biomet and Nemours Foundation.

References

1. Betz RR, D'Andrea LP, Mulcahey MJ, et al. Vertebral body stapling procedure for the treatment of scoliosis in the growing child. Clin Orthop Relat Res 2005;(434):55–60. [PubMed: 15864032]
2. Betz RR, Kim J, D'Andrea LP, et al. An innovative technique of vertebral body stapling for the treatment of patients with adolescent idiopathic scoliosis: a feasibility, safety, and utility study. Spine 2003;28:S255–265. [PubMed: 14560201]
3. Braun JT, Akyuz E, Udall H, et al. Three-dimensional analysis of 2 fusionless scoliosis treatments: a flexible ligament tether versus a rigid-shape memory alloy staple. Spine 2006;31:262–268. [PubMed: 16449897]
4. Braun JT, Hoffman M, Akyuz E, et al. Mechanical modulation of vertebral growth in the fusionless treatment of progressive scoliosis in an experimental model. Spine 2006;31:1314–1320. [PubMed: 16721292]
5. Newton PO, Farnsworth CL, Faro FD, et al. Spinal growth modulation with an anterolateral flexible tether in an immature bovine model: disc health and motion preservation. Spine 2008;33:724–733. [PubMed: 18379398]
6. Newton PO, Faro FD, Farnsworth CL, et al. Multilevel spinal growth modulation with an anterolateral flexible tether in an immature bovine model. Spine 2005;30:2608–2613. [PubMed: 16319746]
7. Newton PO, Fricka KB, Lee SS, et al. Asymmetrical flexible tethering of spine growth in an immature bovine model. Spine 2002;27:689–693. [PubMed: 11923660]
8. Newton PO, Upasani VV, Farnsworth CL, et al. Spinal growth modulation with use of a tether in an immature porcine model. J Bone Joint Surg Am 2008;90:2695–2706. [PubMed: 19047716]
9. Smith AD, Von Lackum WH, Wylie R. An operation for stapling vertebral bodies in congenital scoliosis. J Bone Joint Surg Am 1954;36:342–348. [PubMed: 13152142]
10. Wall EJ, Bylski-Austrow DI, Kolata RJ, et al. Endoscopic mechanical spinal hemiepiphysiodesis modifies spine growth. Spine 2005;30:1148–1153. [PubMed: 15897828]
11. Zhang H, Sucato DJ. Unilateral pedicle screw epiphysiodesis of the neurocentral synchondrosis. Production of idiopathic-like scoliosis in an immature animal model. J Bone Joint Surg Am 2008;90:2460–2469. [PubMed: 18978416]
12. de Jonge T, Slullitel H, Dubousset J, et al. Late-onset spinal deformities in children treated by laminectomy and radiation therapy for malignant tumours. Eur Spine J 2005;14:765–771. [PubMed: 16012842]
13. Katzenstein HM, Kent PM, London WB, et al. Treatment and outcome of 83 children with intraspinal neuroblastoma: The Pediatric Oncology Group experience. J Clin Oncol 2001;19:1047–1055. [PubMed: 11181668]
14. Paulino AC, Fowler BZ. Risk factors for scoliosis in children with neuroblastoma. Int J Radiat Oncol Biol Phys 2005;61:865–869. [PubMed: 15708267]
15. Dodge GR, Bowen JR, Oh CW, et al. Electrical stimulation of the growth plate: a potential approach to an epiphysiodesis. Bioelectromagnetics 2007;28:463–470. [PubMed: 17492657]
16. Dickson RA. Aetiology of idiopathic spinal deformities. Arch Dis Child 1985;60:508–511. [PubMed: 4015167]
17. Dickson RA. The aetiology of spinal deformities. Lancet 1988;1:1151–1155. [PubMed: 2896969]
18. Guo X, Chau WW, Chan YL, et al. Relative anterior spinal overgrowth in adolescent idiopathic scoliosis—result of disproportionate endochondral-membranous growth? Summary of an electronic focus group debate of the IBSE. Eur Spine J 2005;14:862–873. [PubMed: 16133084]

19. Guo X, Chau WW, Chan YL, et al. Relative anterior spinal overgrowth in adolescent idiopathic scoliosis. Results of disproportionate endochondral-membranous bone growth. J Bone Joint Surg Br 2003;85:1026–1031. [PubMed: 14516040]
20. Brighton CT, Shaman P, Heppenstall RB, et al. Tibial nonunion treated with direct current, capacitative coupling, or bone graft. Clin Orthop Relat Res 1995;(321):223–234. [PubMed: 7497673]
21. Goh JC, Bose K, Kang YK, et al. Effects of electrical stimulation on the biomechanical properties of fracture healing in rabbits. Clin Orthop Relat Res 1988;(233):268–273. [PubMed: 3261217]
22. Hodges SD, Jason CE, Humphreys. Use of Electrical Bone Stimulation in Spinal Fusion. J Am Acad Orthop Surg 2003;(11):81–88. [PubMed: 12670134]
23. Brighton CT, Townsend PF. Increased cAMP production after short-term capacitively coupled stimulation in bovine growth plate chondrocytes. J Orthop Res 1988;6:552–558. [PubMed: 2837556]
24. Bozic KJ, Glazer PA, Zurakowski D, et al. In vivo evaluation of coralline hydroxyapatite and direct current electrical stimulation in lumbar spinal fusion. Spine 1999;24:2127–2133. [PubMed: 10543011]
25. Dejardin LM, Kahanovitz N, Arnoczky SP, et al. The effect of varied electrical current densities on lumbar spinal fusions in dogs. Spine J 2001;1:341–347. [PubMed: 14588312]
26. Wang W, Wang Z, Zhang G, et al. Up-regulation of chondrocyte matrix genes and products by electric fields. Clin Orthop Relat Res 2004;(427 Suppl):S163–173. [PubMed: 15480061]
27. Armstrong PF, Brighton CT. Failure of the rabbit tibial growth plate to respond to the long-term application of a capacitively-coupled electrical field. J Orthop Res 1986;4:446–451. [PubMed: 3783299]
28. Okihana H, Shimomura Y. Effect of direct current on cultured growth cartilage cells in vitro. J Orthop Res 1988;6:690–694. [PubMed: 3404326]
29. Sato O, Akai M. Effect of direct-current stimulation on the growth plate. In vivo study with rabbits. Arch Orthop Trauma Surg 1990;109:9–13. [PubMed: 2344269]
30. Forgon M, Vamhidy V, Kellenyi L. Bone growth accelerated by stimulation of the epiphyseal plate with electric current. Arch Orthop Trauma Surg 1985;104:121–124. [PubMed: 3876815]
31. Fredericks DC, Smucker J, Petersen EB, et al. Effects of direct current electrical stimulation on gene expression of osteopromotive factors in a posterolateral spinal fusion model. Spine 2007;32:174–181. [PubMed: 17224811]

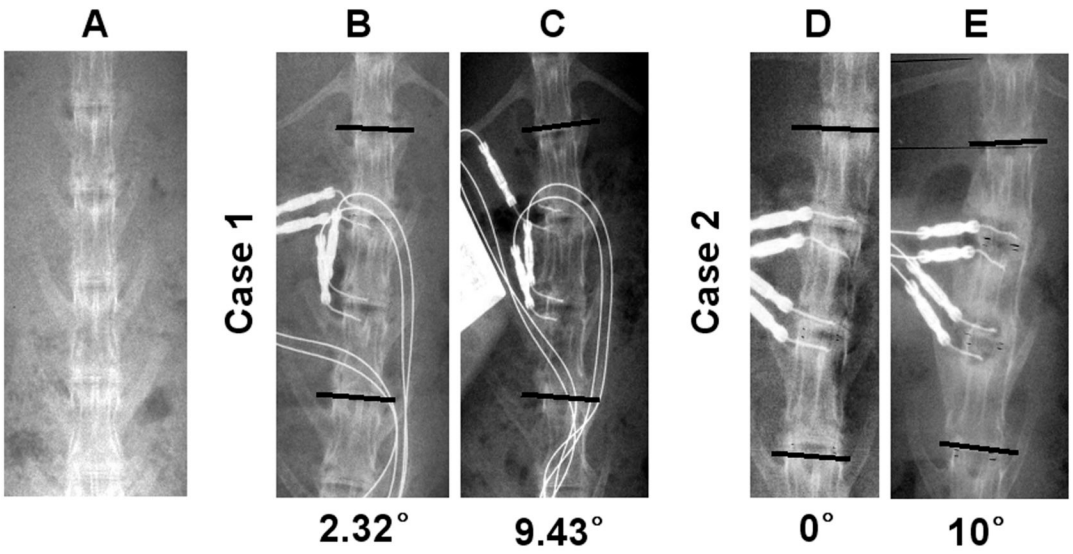

Figure 1.

Two representative cases showing radiographic changes in the untreated normal (A) and treated (B, C, D, E) rabbit spines. AP radiographs are shown of the vertebral after 1 week (B, D) and 6 weeks (C, E) of continuous electrical stimulation at 50μA. The position of the electrodes in the vertebral endplates are also shown. Note the characteristic curve that developed away from the aspect of the vertebrae where the electrodes are placed.

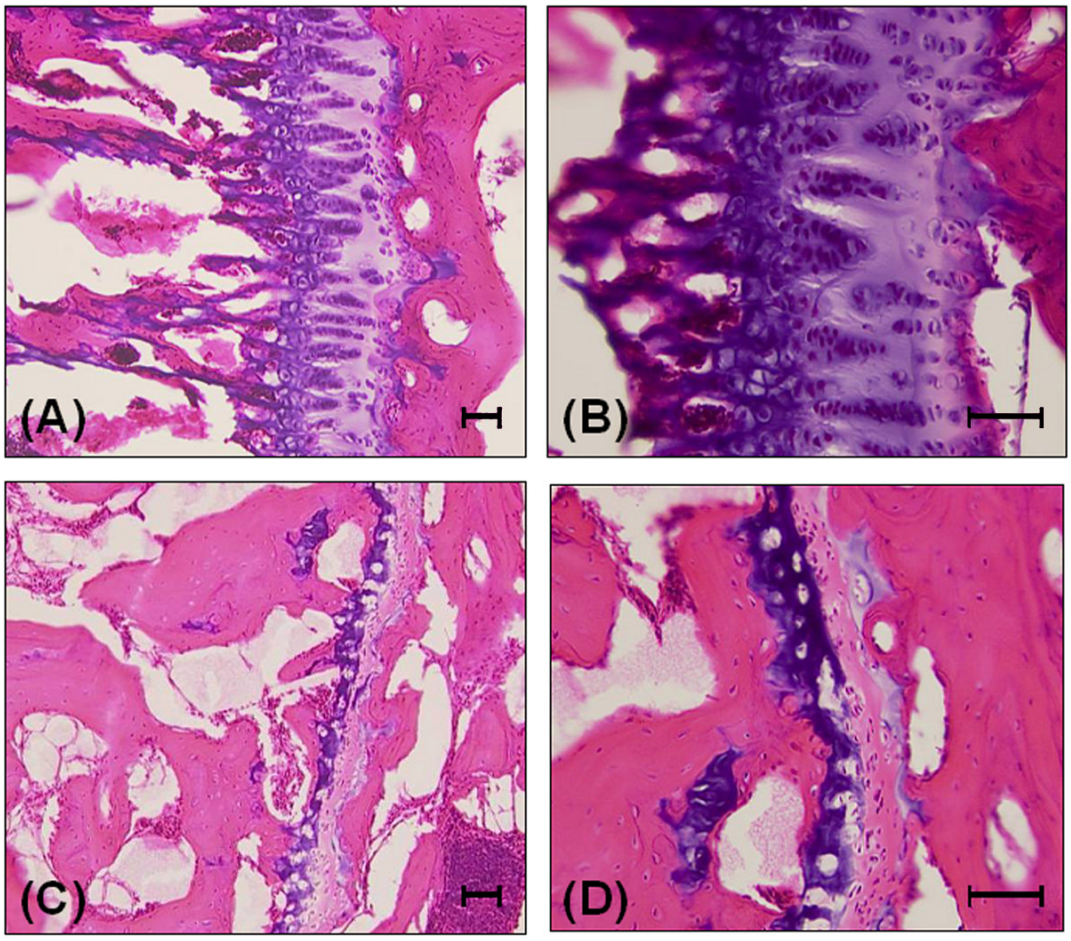

Figure 2.
Narrowing of vertebral endplates after electrical stimulation. Sections are representative of all animals. Sections were stained with Safranin-O. Shown are sections of control untreated vertebrae (A, B) and sections from animals those treated with continuous current at 50 μA for six-weeks (C, D). To demonstrate the height differences and organizational irregularity in growth plate, low (10×) (A, C) and higher magnification (20×) images are shown (B, D). Scale bars = 100 Micron.

Table 1

Table 1. Vertebral height discrepancies were determined by digitizing the radiographs. The height of each vertebral bodies (L1, L2, L3) was measured and the discrepancy of lateral and medial vertebral body heights was determined and is presented. The data shows the growth was consistently less in the operated side in all animals. (* = P value ≥ 0.05).

Group	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0	0.11	0.08	0.05	0.45	0.24	0.25
1-L	2.32*	3.47*	3.34*	3.25*	2.09	5.25*
1-U	0.69	1.18	2.84	3.53	3.92	2.69
2	1.87	3.36*	4.12*	2.69	4.42	3.95*

Group 0: vertebra no electrode

Group 1-L: vertebra + one electrode located at lower physis

Group 1-U: vertebra + one electrode located at upper physis

Group 2: vertebra + two electrodes located at upper and lower physis

Table 2

Changes in the Cobb angle between L1 to L3 at weekly intervals over the 6-week treatment period. The radiographic angular changes were measured at each time point and the mean is presented for all six animal. Blanks indicate no measurement due to animal was terminated early.

Rabbit	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
31676	3.56	1.5	4.7	2.89		
31677	6.01	2.41	10.46	4.32	4.99	6.92
38991	5.27	3.94	6.53	12.74	12.35	6.93
31678	7.73	11.73	8.49	8.11	8.46	9.43
31696	0	7.93	2.63	7.74	12.34	10
38995	0	7.68	7.51	12.53	7.56	8.7
mean	3.761	5.87	6.72	8.05	9.14	8.4