

Anti-TGF- β 2 Antibody Therapy Inhibits Postoperative Resynostosis in Craniosynostotic Rabbits

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Background: Postoperative resynostosis is a common clinical finding. It has been suggested that an overexpression of transforming growth factor (TGF)- β 2 may be related to craniosynostosis and may contribute to postoperative resynostosis. Interference with TGF- β 2 function with the use of neutralizing antibodies may inhibit resynostosis. The present study was designed to test this hypothesis.

Methods: New Zealand White rabbits with bilateral coronal suture synostosis were used as suturectomy controls (group 1, $n = 9$) or given suturectomy with nonspecific, control immunoglobulin G antibody (group 2, $n = 9$) or suturectomy with anti-TGF- β 2 antibody (group 3, $n = 11$). At 10 days of age, a 3×15 -mm coronal suturectomy was performed. The sites in groups 2 and 3 were immediately filled with 0.1 cc of a slowly resorbing collagen gel mixed with either immunoglobulin G (100 μ g per suture) or anti-TGF- β 2 (100 μ g per suture). Three-dimensional computed tomography scan reconstructions of the defects were obtained at 10, 25, 42, and 84 days of age, and the sutures were harvested for histomorphometric analysis.

Results: Computed tomography scan data revealed that the suturectomy sites treated with anti-TGF- β 2 showed significantly ($p < 0.05$) greater areas through 84 days of age compared with controls. Histomorphometry also showed that suturectomy sites treated with anti-TGF- β 2 had patent suturectomy sites and more fibrous tissue in the defects compared with sites in control rabbits and had significantly ($p < 0.001$) less new bone area (by approximately 215 percent) in the suturectomy site.

Conclusions: These data support the initial hypothesis that interference with TGF- β 2 function inhibited postoperative resynostosis in this rabbit model. They also suggest that this biologically based therapy may be a potential surgical adjunct to retard postoperative resynostosis in infants with craniosynostosis. (*Plast. Reconstr. Surg.* 119: 1200, 2007.)

Craniosynostosis is a pathological condition defined as the premature fusion of the sutures of the skull (premature relative to the cessation of brain growth by approximately 6 years of age).¹ The birth prevalence of craniosynostosis is estimated at 300 to 500 per

1,000,000 live births.² Premature suture fusion is associated with secondary deformities in the cranial vault, cranial base, and midface.²⁻⁷ Such skeletal deformities often result in significantly elevated intracranial pressure,⁸⁻¹⁶ altered intracranial volume,^{12,14,17,18} and dilation of the subarachnoid spaces,^{19,20} which may result in optic nerve compression, papilledema, and, if left uncorrected, optic atrophy, blindness,²¹ cogni-

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tive disabilities, and mental retardation.^{8,12,22–25} Such severe craniofacial, ocular, and neural abnormalities can lead to extensive, costly, and often recurrent clinical and surgical management problems.^{24,26–34}

The goals of current therapy and surgical management for craniosynostosis are (1) to provide adequate intracranial volume to allow for normal growth and development, (2) to reestablish normal intracranial fluid pressure dynamics, and (3) to correct the progressive cosmetic skeletal deformity.^{24,26–34} The timing and sequence of these procedures will vary according to the number of affected sutures, the severity of the secondary deformities, and the functional and psychological needs of the patient. Whereas surgical therapies have been successful in achieving these goals, the suture frequently resynostoses,^{32,35–41} especially in cases with simple suturectomies or linear craniectomies.^{35,41} Pancraniosynostosis has even been reported following surgical correction for single-suture synostosis.⁴² This rapid synostosis can increase intracranial pressure and further restrict the growing brain and cranial base.^{11,16,43} These major complications can require additional surgical procedures to correct. Reoperation rates for these procedures have been reported that range from 6 percent to 27 percent.^{24,30,32–34,40,44–46} Such additional operations increase patient morbidity and mortality rates.

Although rapid advances have been made in identifying genetic mutations responsible for craniosynostosis,^{47–51} the pathogenesis of simple, nonsyndromic craniosynostosis appears to be multifactorial and is not completely understood.^{47–51} Recent research has shown that normal suture maintenance and eventual fusion require soluble, heparin-binding growth factors secreted by dura mater.^{35,51–57} One such group of local growth factors that control osteogenic processes in cranial sutures are the transforming growth factor (TGF)- β isoforms (TGF- β 1, TGF- β 2, and TGF- β 3).^{35,51–70} The TGF- β s are potent growth regulatory molecules that influence craniofacial development in early embryonic stages and in subsequent stages of cell differentiation. The activated isoforms are particularly important in suture biology, since they mediate proliferation and differentiation of osteoblastic suture cells and affect fusion in vitro and in vivo.^{35,51–60,62–67,71} Recently it has been shown that mutations in TGF- β receptors 1 and 2 can cause craniosynostosis⁵⁸ and that abnormal expression patterns of these growth factors (especially an overexpression of TGF- β 2)

were noted in craniosynostotic infants^{60,61} and rabbits.^{51,64,65} Such abnormal expression patterns are thought to contribute to premature suture fusion.^{35,51,52,54,59,60,62–65,72} Experimental manipulations of these isoforms in normal rodent sutures have shown that an inhibition of all TGF- β isoforms using a dominant negative TGF- β receptor delayed the fusion of the mouse posterior frontal suture in vitro.^{66,70} It was also shown that inhibition of TGF- β 2 using neutralizing antibodies rescued normally fusing rodent sutures from obliteration.^{59,61,67}

Recent advances in our understanding of the molecular events occurring during normal suture fusion and craniosynostosis,^{4,35,51,57} combined with novel techniques developed to engineer craniofacial tissues,^{51,73–75} may allow us to design targeted and complementary molecular and gene-based therapies to treat or reverse prematurely fusing sutures and decrease complications inherent in high-risk surgical procedures.^{24,30,32–34,40,44–46} The present study was designed to test the hypothesis that inhibition of TGF- β 2 function through the use of neutralizing antibodies inhibits postoperative resynostosis following suturectomy in a rabbit model of human, nonsyndromic craniosynostosis.

MATERIALS AND METHODS

Sample

Twenty-nine, 10-day-old New Zealand White rabbits (*Oryctolagus cuniculus*) with bilateral coronal suture synostosis were used in the present study (Fig. 1). All rabbits were born in our breeding colony of congenitally synostosed rabbits at the University of Pittsburgh's Department of Anthropology vivarium. Morphologically, the synostosed rabbits from this colony are very similar to human infants with congenital bicoronal craniosynostosis. Phenotypically, these rabbits show bony bridging at the coronal sutures as early as 21 days' gestation, obliterated coronal sutures at birth, coronal ridging and brachycephalic cranial vaults by 10 days of age, and secondary changes in the cranial base, brain, and intracranial volume by 42 days of age (Fig. 1).^{6,10,76}

The rabbits were randomly assigned to three groups as follows: group 1, suturectomy with no treatment, which served as the surgical control group ($n = 9$); group 2, suturectomy with non-specific, control immunoglobulin (Ig) G antibody in a slow-release collagen vehicle, which served as the antibody control group ($n = 9$); and (3) suturectomy with anti-TGF- β 2 antibody in a slow-

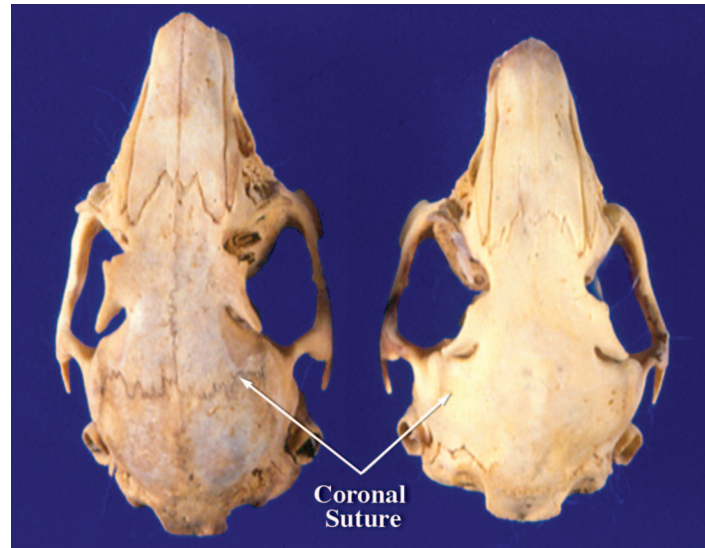


Fig. 1. Cleaned and dried skulls from 42-day-old wild-type (*left*) and biconorally synostosed (*right*) rabbits. Note the fused coronal sutures and brachycephaly of the synostosed skull.

release collagen vehicle, which served as the treatment group ($n = 11$). This study was reviewed and approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

Surgery

At 10 days of age, all rabbits were anesthetized with an intramuscular injection (0.59 ml/kg) of Ketaset (ketamine hydrochloride; Aveco Co., Inc., Fort Dodge, Iowa) and Rompun (xylazine; Mobay Corp., Shawnee, Kans.). The scalps were then shaved, depilated, and prepared for surgery. The calvaria was exposed using a midline scalp incision, and the skin was reflected laterally to the supraorbital borders. Holes were then made in the periosteum and bone using a fine dental bur (0.5 mm) and packed with silver dental amalgam to serve as radio-opaque markers. The holes were placed in quadrants, 2 mm anterior and posterior to the coronal sutures and 2 mm lateral to the sagittal and interfrontal sutures (Fig. 2, *above, left*). All animals received postoperative intramuscular injections (2.5 mg/kg) of Baytril (Bayer Corp., Shawnee Mission, Kans.) as a prophylaxis for infection. After marker implantation, a 3×15 -mm strip of frontal and parietal bones, including the entire length and width of the synostosed coronal suture, was extirpated and removed in one piece from pterion to pterion (Fig. 2, *above, right*) using a cutting burr. Care was taken to preserve the meningeal (fibrous) layer of the dura, the regional

vascularity, and the amalgam markers in the frontal and parietal bones.

In rabbits in the suturectomy control group, only the suturectomy was performed. The periosteal and skin incisions were then closed with 4-0 resorbable Vicryl suture (Ethicon, Somerville, N.J.). In rabbits in the other two groups, the suturectomy sites were immediately filled with 0.1 cc of a slow-resorbing collagen gel mixed with either IgG antibody (100 μ g per suture) or a TGF- β 2-neutralizing antibody (100 μ g per suture). The collagen vehicle was a highly purified, slow-resorbing (>63 days in rabbit perisutural tissues^{50,56}), bovine collagen type I gel and was provided by NeuColl, Inc., (Campbell, Calif.). The gel is approved by the U.S. Food and Drug Administration for human subdermal application and supplied at a density of 65 mg/ml, which is much higher than the density of other collagen gels.^{51,59,77} The gel is diluted to 1:1 with the antibody solution. The IgG and anti-TGF- β 2 antibodies were commercially available (R&D Systems, Minneapolis, Minn.). The antibodies were mixed, under sterile conditions, with 100- μ l aliquots of the collagen gel to a final concentration of 100 μ g per gel aliquot in a 1-ml syringe. The final concentration of the antibody in the collagen gel was 100 μ g/100 ml (32 mg/ml), with 100 μ g per suture. This volume assured that the entire suturectomy site was filled with vehicle and antibody (Fig. 2, *below, left and right*). After injections, the periosteum and skin incisions were closed with 4-0 resorbable Vicryl

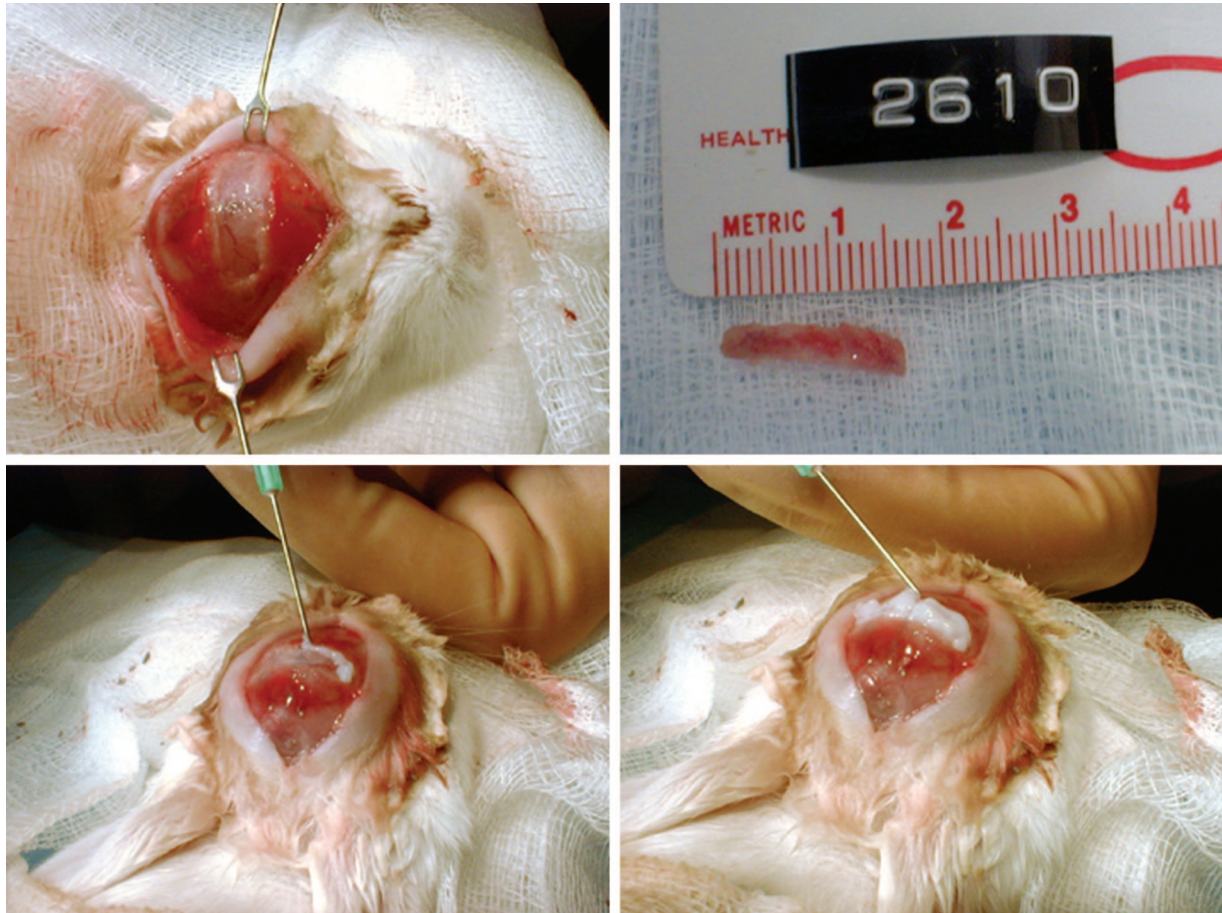


Fig. 2. Surgical sequence showing the coronal suturotomy site (*above, left*), the excised coronal suture (*above, right*), and the placement of the collagen vehicle into the suturotomy site (*below, left and right*).

suture (Ethicon). Optimal anti-TGF- β 2 antibody dosage and biodegradation kinetics of the collagen vehicle were determined *in vitro* and presented elsewhere by one of our coauthors (A.M.M.).^{51,59,77}

Body Weights and Computed Tomography

Body weight and three-dimensional head computed tomographic scan data of the suturotomy sites were obtained from all rabbits at 10, 25, 42, and 84 days of age, an age at which approximately 80 to 90 percent of total calvarial and brain growth is completed in the rabbit.^{6,76,78}

Serial body weights were taken with a Tanita digital scale (NLS Animal Health, Baltimore, Md.). Standardized, serial three-dimensional computed tomographic scans^{17,18} were taken with the rabbits tranquilized with an intramuscular injection (0.40 ml/kg) of a solution of 91% Ketaset (ketamine hydrochloride, 100 mg/ml) and 9% Rompun (xylazine, 20 mg/ml). The rabbits were all scanned in the sagittal plane using a GE

HiSpeed Advantage Scanner with identical settings (DFOV, 24.0 to 18.0 cm; mA, 120 to 150; kV, 120) at a thickness of 1 mm. The suturotomy site was traced manually and reconstructed, and the remaining defect area was calculated using Allegro Software (ISG Technologies, Atlanta, Ga.) on a Sun Workstation.^{17,76} All measurements were taken blind as to rabbit group identity, and intraobserver repeated measurement reliability was calculated ($r = 0.941$; $p < 0.01$) on a randomly drawn sample (20 percent) of rabbits.

Histomorphometry

At 84 days of age (74 days postoperatively), the rabbits were euthanized with an intravenous (40 mg/kg) injection of pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.), and the suturotomy sites and adjacent parietal and frontal bones were harvested for histological examination (specimens were approximately 6 mm long and 15 mm wide). The specimens were fixed in 10% buffered neutral formalin, demineralized in

a formic acid solution (Calex II, Fisher Scientific, Chicago, Ill.), dehydrated in a series of alcohol washes, and embedded in paraffin. The specimens were sectioned in the sagittal plane in the middle of both the right and left coronal sutures at a thickness of 5 to 7 μm . The middle of each coronal suture was chosen for analysis because it is the focal point of synostosis in these rabbits. The synostosis then normally progresses in both medial and lateral directions.^{76,79} Thus this area is considered the most osteogenic and should be most affected by molecular manipulation. For each right and left side of the suturectomy site, three sections were stained at 30- μm intervals with hematoxylin and eosin for conventional, qualitative, bright-field light microscopy and histomorphometric analysis.

Histomorphometry of the area of total new bone in the suturectomy site was performed using a Leica MZ12 Stereo Zoom microscope and Northern Eclipse (version 5.0) Image Analysis Software (Empix Imaging, Inc., New York, N.Y.). The original suturectomy site margins were identified, and the boundaries of only the regenerated, new bone in the suturectomy site were traced manually from the digital images and the area calculated. If there were multiple osseous islands in the suturectomy site, all the bony islands were measured and the sum of the areas was used. All measurements were taken blindly with regard to group identity, and a random sample of 20 percent of the specimens was measured twice by the same individual. Intrarater reliability was $r = 0.999$ ($p < 0.001$).

Statistical Analysis

Means and standard errors of the mean for body weight and suturectomy site area from three-dimensional computed tomographic scans at each age were calculated and compared among ages and groups using a two-way analysis of variance with repeated measures. Mean total new bone area from the histological specimens at 84 days was calculated and compared among groups using a one-way analysis of variance. Significant intergroup differences were assessed using the least significant differences multiple comparison test. All data were analyzed using SPSS 12.0 for Windows (SPSS, Inc., Chicago, Ill.). Differences were considered significant if the p value was less than 0.05.

RESULTS

Somatic Growth and Coronal Suturectomy Site Patency

Mean body weight in all three groups of rabbits increased similarly from approximately 0.2 kg at 10 days of age to 1.7 kg at 84 days of age (Fig. 3). A significant age ($F = 198.18$; $p < 0.001$) main effect was noted, while no significant group ($F = 0.47$; not significant) or group \times age interaction effects ($F = 0.45$; not significant) were noted.

Serial three-dimensional computed tomographic reconstructions (Figs. 4 and 5) showed very rapid reossification of the coronal suturectomy site in the suturectomy control group and, to a lesser extent, in the IgG control group compared with the anti-TGF- β 2 group by 42 days of age (32

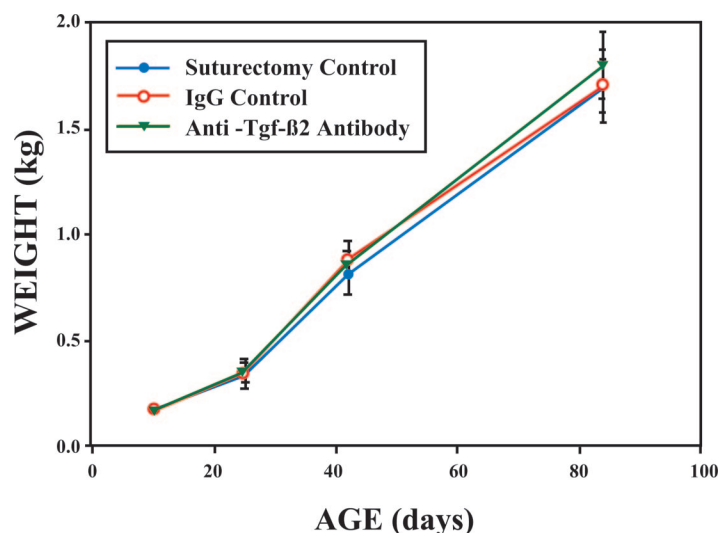


Fig. 3. Mean body weight (\pm SE). No differences were noted among groups.

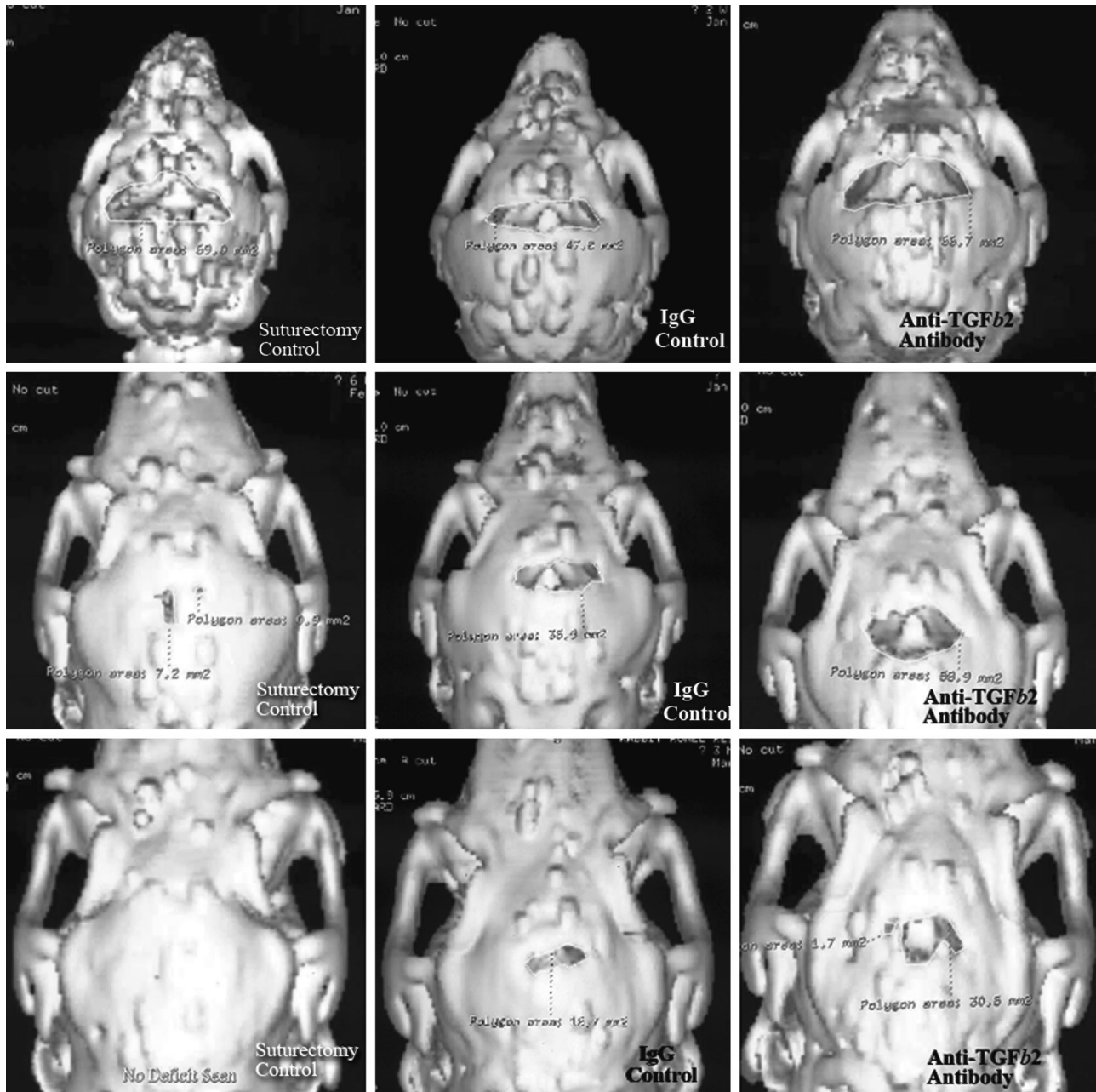


Fig. 4. Superior three-dimensional computed tomographic scan reconstructions of the suturotomy sites from the three groups under study at 10 (above), 42 (center), and 84 (below) days of age. Note the resynostosis in the suturotomy and IgG control groups compared with that in the anti-TGF- β 2 antibody-treated group.

days postoperatively). The sites showed extensive new bone formation at the margins of the suturotomy that eventually resynostosed in the control groups by 84 days of age (74 days postoperatively) (Figs. 4 and 5). In contrast, rabbits receiving postoperative anti-TGF- β 2 antibody therapy showed some resynostosis and bony bridging of the suturotomy site by 84 days of age, but most rabbits still had sizeable, patent defects at this time. The

three-dimensional computed tomographic scan reconstructions also showed that the intracranial contents were longer in the anteroposterior dimension and less superiorly displaced in the anti-TGF- β 2 antibody group compared with the both the suturotomy and IgG control groups by 84 days of age (Fig. 5).

The mean suturotomy site area in both the suturotomy and IgG control groups decreased

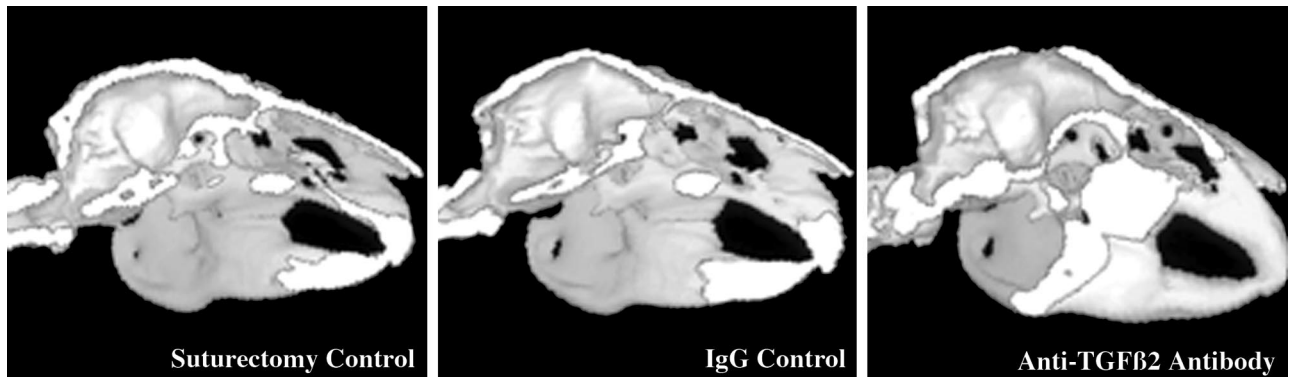


Fig. 5. Lateral three-dimensional computed tomographic scan reconstructions of the suturotomy sites from the three groups under study at 84 days of age. Note the resynostosis and intracranial growth restrictions in the suturotomy and IgG control groups compared with those in the anti-TGF- β 2 antibody-treated group.

rapidly through 84 days of age, leaving only approximately 25 percent of the defect patent by this time, as measured from the three-dimensional computed tomographic scan reconstructions (Fig. 6). IgG control rabbits showed slightly slower reossification than the suturotomy control rabbits did at 25 and 42 days of age (Fig. 6). In contrast, the suturotomy site in rabbits in the anti-TGF- β 2 group showed minimal reossification in the first 2 weeks postoperatively, and approximately 70 percent of the defect was still patent by 84 days of age, paralleling the reossification pattern in both the suturotomy and IgG control groups through 84 days of age (Fig. 6). Two-way analysis of variance revealed significant group ($F = 22.68$; $p < 0.001$) and age ($F = 37.07$; $p < 0.001$) main effects. No

significant group \times age interaction ($F = 0.14$; NS) was noted. Multiple comparison tests revealed that at all ages, mean suturotomy area was significantly ($p < 0.05$) greater in the anti-TGF- β 2 group compared with the other two control groups. No significant mean differences ($p > 0.05$) were noted between the two control groups at any age (Fig. 6).

Suturotomy Site Resynostosis and Histomorphometry

At 84 days of age, suturotomy and IgG-treated control suturotomy sites had extensive reossification and resynostosis in the suturotomy sites (Fig. 7), as seen with bright-field light microscopy.

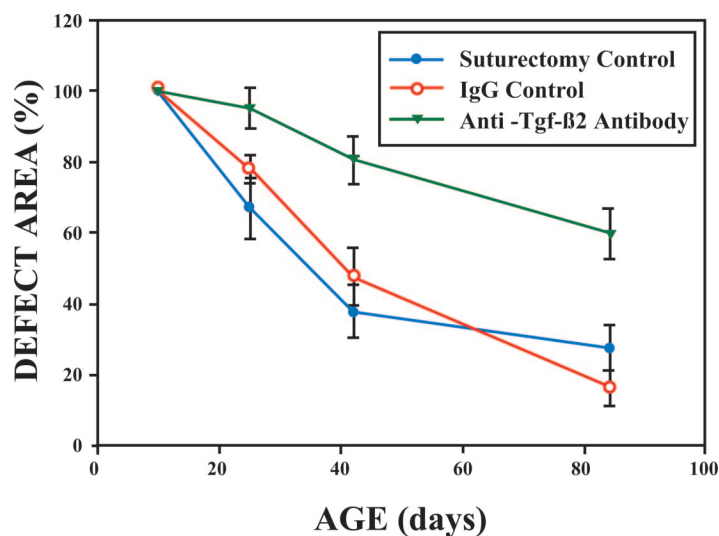


Fig. 6. Reossification of the coronal suturotomy site (mean \pm SE area) by group and age. Note the delayed healing in the anti-TGF- β 2 antibody-treated group compared with the suturotomy and IgG control groups.

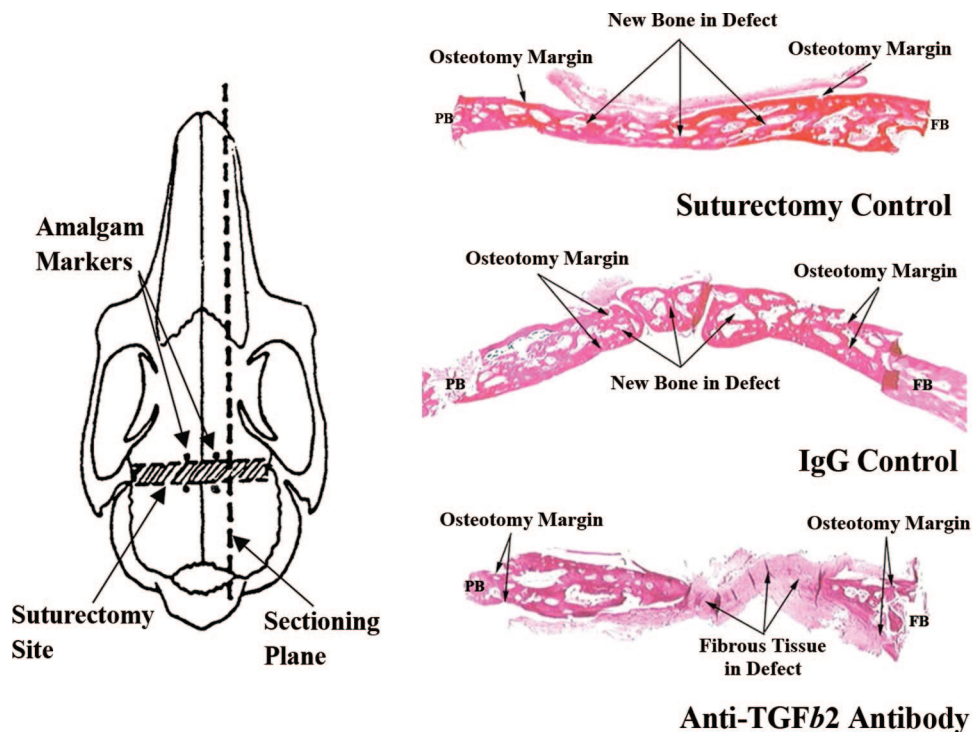


Fig. 7. Diagram showing suturotomy site harvesting, orientation of histological sectioning in the sagittal plane, and tissue specimens of the suturotomy sites from the three groups at 84 days of age. Note the extensive reossification of the suturotomy sites in the suturotomy and IgG control groups compared with the anti-TGF- β 2 antibody–treated group (original magnification, 25 \times). PB, parietal bone; FB, frontal bone.

The new bone was seen to extend both anteriorly from the parietal bones and posteriorly from the frontal bones and form extensive bridging throughout the original suturotomy site. The bone was similar in morphology to the bone in the original suturotomy site margins, and it was extensively thickened on the endocranial (dural) surface, with increased staining of osteoblasts along the surface and osteocytes in the lacunae. Most of the bone in the suturotomy site was fused in the middle of the defect and showed no evidence of a coronal suture (patent or obliterated). In contrast, suturotomy sites treated with anti-TGF- β 2 antibody showed much less reossification and resynostosis than controls did. The suturotomy site margins were thinner, had more tapered edges, and showed extensive fibrous tissue formation in the original suturotomy sites compared with controls (Fig. 7).

Quantitative evaluation of the suturotomy sites showed that suturotomy and IgG-treated control groups had an average of 2.8 mm² of total new bone in the suturotomy site compared with an average of 0.7 mm² of new bone in the suturotomy sites of rabbits treated with anti-TGF- β 2 antibody (Fig. 8), an increase of approximately 215

percent more new bone in the control rabbits compared with rabbits treated anti-TGF- β 2 antibody. One-way analysis of variance revealed that rabbits treated with anti-TGF- β 2 antibody therapy

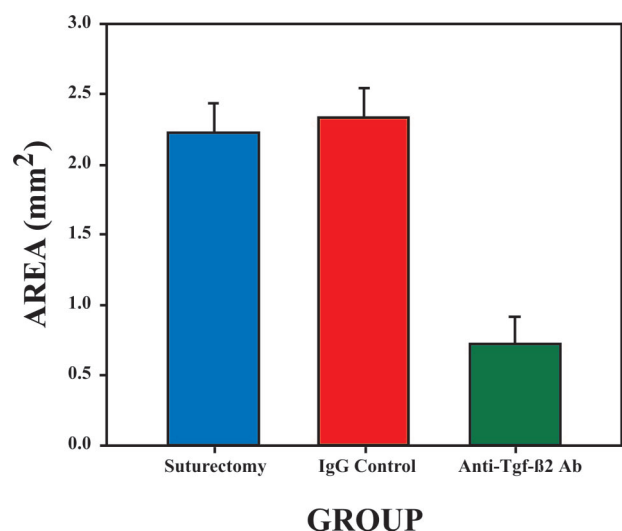


Fig. 8. New bone at suturotomy margins (mean \pm SE area) by group. Note the extensive new bone found in the suturotomy sites in the suturotomy and IgG control groups compared with the anti-TGF- β 2 antibody–treated group at 84 days of age.

had significantly less ($F = 15.16$; $p < 0.001$) new bone in the suturectomy site than the other two control groups did. No significant mean differences ($p > 0.05$) in new bone formation were noted between the two control groups (Fig. 8).

DISCUSSION

The present study demonstrates that a course of treatment with TGF- β 2-neutralizing antibodies in a slow-release collagen vehicle following surgical release of a prematurely fused coronal suture results in significantly reduced reossification of the suturectomy site compared with controls in a craniosynostotic rabbit model. These data are consistent with in vitro data showing inhibition of normal rodent suture fusion by interfering with TGF- β 2 function.^{35,51,57,59,67} However, before the end of the rabbit neurocranial growth phase (84 days of age), resynostosis was still seen in the anti-TGF- β 2 antibody-treated group, suggesting that the effect of this antibody therapy delivered via a resorbable collagen vehicle may only be transitory.

Although it is still unclear how the different TGF- β isoforms function to regulate suture patency and fusion, it has been shown that each isoform binds to and signals through the same set of receptors.^{51-53,57,68-70} Blocking TGF- β 2-binding activity and function using neutralizing antibodies may allow TGF- β 3 to bind to its receptor without competition, thus obviating the antagonistic effects of these two isoforms in regulating postoperative resynostosis.^{35,51,57,63,66-69} TGF- β s may also regulate postoperative resynostosis in much the same way that they regulate suture fusion, by controlling the number of cells in the suture and surrounding bone fronts by regulating cell proliferation and apoptosis.^{51,57,67,69} Postoperative resynostosis in rabbits from this colony may be a result of increased cell proliferation rates from TGF- β 2 overexpression, as evidenced by the significantly increased number of osteoblasts and osteocytes and new bone area seen in the suturectomy margins in control rabbits compared with anti-TGF- β 2 antibody-treated rabbits in this study. These findings are supported by data from Sanford et al.,⁸⁰ who show calvarial bone dysgenesis and sutural agenesis in TGF- β 2 knockout mice. Since all three of the TGF- β isoforms are present in the perisutural tissues, it is likely that there is a complex interplay among them and their receptors, other growth factors (e.g., fibroblast growth factors and bone morphogenetic proteins), and various developmental genes (e.g., *MSX2*, *TWIST*, and *RUNX2*) in regulating suture pa-

tency, which may also be involved in postoperative resynostosis.^{4,35,49-52,57,63,65}

The goals of current therapy and surgical management of craniosynostosis are to provide adequate intracranial volume to allow for normal brain growth and development,^{8,12,17-20,22-25} to re-establish normal intracranial fluid pressure dynamics,⁸⁻¹⁶ and to correct the progressive cosmetic skeletal deformity.²⁻⁷ The timing and sequence of these procedures will vary according to the number of affected sutures, the severity of the secondary deformities, and the functional and psychological needs of the patient.^{24,26-35} Surgical management typically involves the following: (1) the surgical release of the synostosed suture, cranial vault decompression, and upper orbital reshaping and advancement in infancy (approximately 3 to 12 months of age); (2) the surgical correction of midfacial deformities in childhood (approximately 4 to 12 years of age); and (3) orthognathic surgery to correct jaw discrepancies in adolescence (approximately 14 to 18 years of age).^{24,26-33} Although these surgical procedures are successful in achieving the goals stated above, the suturectomy site frequently reossifies (in 30 percent to 100 percent of reported cases),^{32,35-41} and this occurs very rapidly (in some cases as early as 6 months postoperatively), especially in sporadic or isolated cases in which simple suturectomies or linear craniectomies have been used.^{35,36,41} Pancraniosynostosis has even been reported following surgical correction for single-suture synostosis.⁴² This rapid resynostosis can increase intracranial pressure, further restrict the growing brain and cranial base, and alter craniofacial growth.^{11,16,18,27,43,51}

To overcome such reossification problems, a number of different clinical strategies have been utilized in the past.^{24,33,44,81,82} Initial attempts at preventing previously released stenosed sutures from closing again involved wrapping the intact bony margins with a barrier. However, new bone rapidly overgrew the barrier and reossified the suturectomy site, and new suture formation was never observed.⁴⁴ In contrast, other clinical investigators have utilized techniques designed to chemically damage the dura and reduce its osteogenic potential.^{44,83,84} The frequency of suturectomy site patency increased with the use of these techniques, but seizures and neurological problems were typically a consequence, probably due to the toxic effects of the chemical adjuvants used.^{24,44} Autotransplantation or allotransplantation of normal sutures into the suturectomy site to replace missing growth sites has also been utilized,

but rapid resynostosis of the transplanted suture was also seen in both human infants⁴¹ and rabbits with familial craniosynostosis.⁴³ Another approach has involved the extensive surgical intervention and radical repositioning of the calvarial bones performed in part to keep the margins of the craniectomy sites from physically reapproximating and resynostosing.^{24,26–35,44,81} Although sutural reossification is less of a problem with these advancement techniques, the rates for other complications from these high-risk procedures range from approximately 15 percent to 25 percent and include osseous relapse and instability, severe intraoperative blood loss, convulsions, infections, conjunctival chemosis/ facial swelling, and soft-tissue necrosis.^{24,29,32,34,40,44–46} The major complications can require additional surgical procedures to correct (reoperation rates range from 6 percent to 27 percent)^{45,46} and are more frequent in infants with syndromic craniosynostosis,¹⁵ which in turn increases patient morbidity and mortality rates.

It was interesting to note that the suturectomy site area in rabbits treated with anti-TGF- β 2 antibody therapy remained stable from 10 to 25 days of age (15 days postoperatively) and then showed a reossification rate similar to that of the other two control groups through 84 days of age. These data suggest that the neutralizing antibodies were functional for about 2 or 3 weeks postoperatively and then were probably degraded during wound healing. Another explanation may be that the available anti-TGF- β 2 antibody may remain functional but simply be overwhelmed by the proliferating osteoblasts and the overexpression and accumulation of TGF- β 2 in the suturectomy site. However, even with this transient effect on resynostosis, the therapeutic regimen still inhibited reossification by approximately 50 percent in anti-TGF- β 2 antibody-treated rabbits compared with controls. It was also interesting to note that the IgG-treated control group showed slightly less mean reossification of the suturectomy site at 25 and 42 days of age compared with the suturectomy controls, although these differences were not statistically significant. This is probably not an effect of the IgG but rather due to the presence of the collagen vehicle itself in the suturectomy site, which may have had an osteoinhibitory effect during degradation. A recent *in vitro* study⁷⁷ has also shown that the collagen vehicle alone had a short-term inhibitory effect on osteoblast cell number in culture. Since the goal of the present study was to inhibit postoperative resynostosis, this should not be viewed as a confounding variable, although stud-

ies designed to facilitate osteogenesis using various growth factors delivered by this collagen vehicle should take this into consideration.

Although results from this rabbit model of craniosynostosis are promising, there are a number of practical problems related to the human use of these biologically based therapies. Brain growth in humans is about 95 percent complete by 6 years of age,¹ compared with 84 days in rabbits. Thus, sutures or surgical sites should theoretically remain patent for at least this long to allow unrestricted brain and neurocapsule growth in human infants. Although there is controversy concerning the timing of primary surgical release (3 to 12 months of age), and reossification in some cases has been seen by 6 months postoperatively, these biologically based therapies will require sustained growth factor release for approximately 5 years to inhibit resynostosis and provide a therapeutic effect clinically. However, in human infants, approximately 60 percent of brain growth is finished by the first year of life and 90 percent is finished by the second year.¹ Thus interference with resynostosis might be more efficacious in the immediate postoperative period during the first year and less so in the second year and beyond. Given the present technology for delivering growth factors, antibodies, and genes,^{51,73–75} multiple dosing will likely be required to deliver the requisite amounts. Vehicles will also have to be designed to achieve this prolonged delivery length in order for a biologically based therapy to be effective. With the increased use of endoscopy in the treatment of craniosynostosis,⁸⁵ these procedures could be performed more conservatively and be used to deliver a molecular or gene-based therapy. Although craniosynostosis is multifactorial and exhibits genetic heterogeneity, hyperostosis and postoperative resynostosis are common clinical correlates of most primary craniosynostosis.⁸⁵ These findings suggest that the same transcription and growth factors and their receptors are involved in similar molecular pathways leading to suture obliteration and resynostosis.^{35,47–51} Thus, even though the genetic mutation responsible for craniosynostosis in this rabbit model has not been identified yet, data obtained from this model suggest that postoperative resynostosis probably utilizes one of a number of highly conserved signaling pathways. This should make the design and development of other biologically based therapies much easier, especially if the goal is to interrupt downstream signaling and reduce osteogenesis at the surgical site and not treat the primary genetic etiology of craniosynostosis.^{35,51}

These findings suggest that much more research is needed to improve the clinical and surgical management and the eventual quality of life for infants born with craniosynostotic defects. The utility of developing biologically based therapies, as an adjunct to earlier and less radical surgical intervention strategies, is evident.⁵¹ This approach should prevent postsurgical resynostosis, reestablish the normal intracranial fluid pressure dynamics, improve craniofacial and neurocapillary growth, and obviate multiple operations for neonates with various synostotic conditions.

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DISCLOSURE

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