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Changes in Biomechanical Strain and Morphology of Rat Calvarial Sutures and Bone after Tgf- β 3 Inhibition of Posterior Interfrontal Suture Fusion

Reiko Shibazaki-Yorozuya^{a,b}, Qian Wang^c, Paul C. Dechow^a, Koutaro Maki^b, and Lynne A. Opperman^a

^aTexas A&M Health Science Center Baylor College of Dentistry, Department of Biomedical Sciences, Dallas, TX, USA

^bDepartment of Orthodontics Showa University School of Dentistry, Tokyo, Japan

^cDivision of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, USA

Abstract

Craniofacial sutures are bone growth fronts that respond and adapt to biomechanical environments. Little is known of the role sutures play in regulating the skull biomechanical environment during patency and fusion conditions, especially how delayed or premature suture fusion will impact skull biomechanics. Tgf- β 3 has been shown to prevent or delay suture fusion over the short term in rat skulls, yet the long-term patency or its consequences in treated sutures is not known. It was therefore hypothesized that Tgf- β 3 had a long-term impact to prevent suture fusion and thus alter the skull biomechanics. In this study, collagen gels containing 3 ng Tgf- β 3 were surgically placed superficial to the posterior interfrontal suture and deep to the periosteum in postnatal day 9 (P9) rats. At P9, P24, and P70, biting forces and strains over left parietal bone, posterior interfrontal suture, and sagittal suture were measured with masticatory muscles bilaterally stimulated, after which the rats were sacrificed and suture patency analyzed histologically. Results demonstrated that Tgf- β 3 treated sutures showed less fusion over time than control groups, and strain patterns in the skulls of the Tgf- β 3 treated group were different from that of the control group. While bite force increased with age, no alterations in bite force were attributable to Tgf- β 3 treatment. These findings suggest that the continued presence of patent sutures can affect strain patterns, perhaps when higher bite forces are present as in adult animals.

Keywords

Cranial sutures; cranial bone; suture fusion; Tgf- β 3; biomechanics

INTRODUCTION

Cranial sutures are the major sites of calvarial bone expansion (Baer, 1954; Opperman, 2000). The form of the cranial vault is modified under the influence of masticatory hypofunction or hyperfunction (Ulgen et al., 1997; Katsaros et al., 2002; Wright, 2005) and growth of the underlying brain (Kreiborg, 2000). On the other hand, in cases such as premature obliteration of sutures (craniosynostosis), the normal pattern of bone growth is altered (Jane and Persing, 1986; Burrows et al., 1995), and often the expansion of cranial bones is limited (Singhal et al., 1997; Mooney et al., 1999).

Although cranial sutures form in the absence of muscle activity (Hirabayashi et al., 1989), a number of studies support Moss' hypothesis that the fine details of suture morphology are secondary responses to extrinsic forces, and incurred strain modes (Moss, 1957; Herring and Mucci, 1991; Anton et al., 1992; Rafferty and Herring, 1999; Byron et al., 2004; Markey et al., 2006; Markey and Marshall, 2007; Byron, 2009). In patent sutures, suture complexity and tissue structure changes over time and with a change in masticatory forces (Massler and Schour, 1951; Gross, 1961; Milch, 1966; Kokich, 1976; Miroue and Rosenberg, 1975; Saito et al., 2002; Mao, 2003; Byron et al., 2004; Wang et al., 2006b; Byron, 2009; Smith et al., 2010). The change in suture morphology before suture fusion (such as bony bridging, interdigitation, and beveling) is thus of particular interest within the context of the biomechanical environment. Masticatory muscle resection and surgical isolation and mechanical immobilization of portions of sutures result in the loss of sutural complexity (Moss, 1954; Koskinen, 1977; Foley and Kokichi, 1980), implying an association between suture patency and craniofacial biomechanics. The effects of mastication on growth of the calvaria have been studied in a variety of mammals (Behrents et al., 1978; Jaslow, 1990; Mao et al., 2003a,b; Rafferty et al., 2003). Average strain values in patent sutures are an order of magnitude higher than in adjacent regions of cortical bone (Behrents et al., 1978; Jaslow, 1990; Rafferty et al., 2003; Lieberman et al., 2004; Wang et al., 2008). Previous research has shown that posterior interfrontal sutures (IFS) and sagittal sutures (SGS) have individual strain patterns that change with age and as they fuse (Shibazaki et al., 2007). Strain patterns are related to suture fusion (Sun et al., 2004), however the question of how suture morphology and fusion affect the global biomechanics of the craniofacial skeleton has not been systematically studied.

Suture fusion is closely associated with changes in gene expression. Mutations in genes for fibroblast growth factor receptors (*FGFRs*), *MSX2*, and *TWIST* have all been associated with premature suture fusion (craniosynostosis) (Jabs, 2002). Mutations in transforming growth factors beta (*TGF-βs*) have not been associated with premature suture fusion. However, recent findings show that mutations in *TGF-β* receptor type II (*TβR-II*) are associated with craniosynostosis (Loeys et al., 2005). *In vitro* data have shown that *Tgf-β2* and *Tgf-β3* are potent regulators of suture patency (Opperman et al., 1999, 2000). *Tgf-β2* added to fetal rat calvariae in culture induced the normally patent coronal sutures to fuse, while *Tgf-β3* rescued these sutures from fusion. Furthermore, IFS obliteration was delayed in the short term by *Tgf-β3 in vivo* (Opperman et al., 2002; Chong et al., 2003). It is possible that *Tgf-β3* prevented the loss of the suture mesenchyme as described in cases of craniosynostosis by Holmes et al. (2009).

While these data show that changes in strain patterns and growth factor expression are related to suture patency, it is important to determine if a change in the status of suture patency (i.e. the process of fusion and suture obliteration) alter the cranial biomechanical environment. The hypothesis to be tested is that *Tgf-β3* effects long-term suture patency, and that preventing suture fusion will alter strain patterns in craniofacial sutures. To test this hypothesis, a rat cranial suture model was used, with three experimental aims. The first aim was to delineate the long-term effects of *Tgf-β3* on degree of IFS bony bridging and suture morphology. The second aim was to determine whether *Tgf-β3* treatment of the IFS had any effect on bite force, and the final aim was to compare the overall strain patterns across patent sagittal sutures (SGS) and fusing or non-fusing IFS and the adjacent parietal bone (PB).

MATERIAL AND METHODS

Animals

Pregnant Sprague-Dawley rats were purchased (Harlan, Indianapolis, IN, USA) and housed in the animal facility at the Baylor College of Dentistry. Of the pups born to these rats, 180

nine-day old (P9) male and female pups were used in this study (Table 1). The protocol for surgery and post-surgical observation and care followed the guidelines dictated by the Baylor College of Dentistry IACUC, and has been described previously (Shibazaki et al., 2007). All animals for this study were housed in the Animal Research Facility at Baylor College of Dentistry.

Surgery

The first author conducted all surgeries, using methods reported previously (Opperman et al., 2002). Nine days after birth (P9), rat pups were anesthetized using 3.25% Isoflurane (Butler, Dallas, TX, USA) and 900 cc/min O₂ inhalant anesthetic delivered by a Foregger F500 anesthesia system via a nose cone. Animals were randomly assigned to four groups (n=45 per group). Group 1 served as non-surgical control (CON). Group 2 was used as a sham surgical control (Sham). In this group, a coronal incision through the scalp was made from ear to ear, the skin was reflected to expose the coronal and IFS, and the periosteum was loosened above the IFS by use of a periosteal elevator. No further manipulation was done prior to closing the incision. Group 3 had bovine collagen type I gel soaked with sterile phosphate-buffered saline (PBS) placed on the exposed IFS beneath the periosteum (Gel only). Group 4 received collagen gels adsorbed with 3 ng Tgf- β 3 (β 3). The two edges of the scalp were approximated and closed with a 6-0 silk suture (DG, New York, NY), with care taken not to damage or move the gel.

At P9, P24, and P70 (0, 15 or 61 days after surgery), rats were anesthetized by intramuscular injection (1 ml/kg body weight) of a solution containing 13 mg Rompun and 87 mg Ketamine in each ml of cocktail prior to strain and bite force measurement. After measurements were taken at each time point, the rats (n=60 per time point) were sacrificed by Halothane (Butler, Dallas, TX, USA) overdose, and tissues prepared for histology.

Histology

Sixty rats not used for bite-force or strain measurements (n=5 per time point per group) were used for histology. They were decapitated and the scalps removed. The heads were postfixed overnight in 4% paraformaldehyde and decalcified using 0.5M EDTA for two weeks (P9, P24) or for one week with microwave (P70). Different decalcification methods were required because of the much thicker P70 bones. Following decalcification, the heads were cut in half along the coronal suture, separating each into two cranial regions, one containing frontal bones with intervening fusing or non-fusing IFS and the other containing PB with intervening patent SGS. Samples were processed for paraffin embedding and sectioning at 6 μ m and three sections from midway through each tissue were stained with hematoxylin and eosin (H&E). Sections were used for qualitative examination of suture morphology, and for scoring the amount of bony bridging within the sutures.

Scoring of bony bridging within the IFS

To examine the patency of each suture, 3 sections from each rat IFS and SGS (n=360 sections total) were stained with H&E, and examined using polarized light microscopy. The SGS was examined to determine if the Tgf- β 3 placed over the IFS had any effect on patency of the SGS. In the cross-sections, the vertical height of the parietal and frontal bones was measured, along with the vertical height of the fused region of the suture and the vertical height of the unfused portion of the suture (Fig. 1A). These measurements were used to calculate a percentage of bony bridging in the suture. It is worth noting that this scoring method was for assessing the degree of sutural fusion along the direction of bone thickness to the effect of Tgf- β 3 or lack of it, not for assessing a correlation between suture morphology (such as interdigitation, beveling, and fusion) and biomechanics; the latter needs a three dimensional image database (Markey and Marshall, 2007). A two-sample t test

(for the two tailed hypotheses) was used to test the differences between the Tgf- β 3 group and the rest of the groups in degrees of bony bridging and suture fusion. The significance level was set at $\alpha = 0.05$.

Masticatory muscle stimulation and bite-force measurement

On the remaining 120 rats (4 groups with $n=30$ animals per group, and $n=10$ for each time point), muscle stimulation was achieved via unipolar needle electrodes (Platinum Subdermal Electrode Type E2, Grass Instrument Co., MA, USA) inserted through the skin bilaterally into each masseter muscle. Under anesthesia, a super-maximal train of pulses (120 Hz, 40 V, single train) was delivered to the muscles by a SIU5 Stimulus Isolation Unit/Solid-State Square Pulse Stimulator (Grass Instrument Co., MA, USA).

The bite force transducer consisted of two differential strain aluminum beams, 4.0 mm in thickness, mounted with four strain gages in a full bridge configuration (Shibazaki et al., 2007). After calibrating the bite force transducer, all bite force measurements were recorded by incisor biting. The strain was calibrated to force (gf) with a conversion chart made by measuring weights. The masseter muscle stimulation was coordinated with both bite force measurements and strain measurements.

IFS/SGS/PB strain measurement

The same rats used for bite-force measurement were used for strain measurement. One strain gage was placed for each rat. After anesthesia, the skin and periosteum were incised over sites chosen for strain gage placement across the IFS or SGS, or on the PB. The exposed bony surfaces were smoothed, degreased and dried as before (Shibazaki et al., 2007). A stacked delta rosette gage (Model UFRA-1-11, Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan) was bonded across the posterior part of the IFS, the anterior part of the SGS or on the PB next to the anterior part of the SGS (Fig. 2). In UFRA models, the backing plate is thinnest and more flexible than in other existing models, which is important for use on curved surfaces such as those on bone. During masseter muscle stimulation, strain signals were recorded, digitized, sampled and stored to a sensor interface (PCD-300A, Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan). Values from each element of the rosette gage were analyzed, and principal maximum (tensile) and minimum (compressive) strain magnitudes and orientations were calculated using application software (DAS-100A, Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan). Tensile strains were expressed as positive values and compressive strains as negative values. Peak strains were selected based on the 3rd stimulation record of the 10th biting cycle of each animal. Orientation of maximum strain (θ max) was presented using Oriana 2.0 software (Kovach Computing Services, Wales, UK).

RESULTS

Suture morphology and degree of fusion within the IFS

At P9, sutures from all groups remained patent (data not shown). At P24, the suture morphology of the Sham, Gel only and β 3 groups were similar to that of CON sutures (Fig. 3). The IFS showed bony bridging from the dural side in all groups where fusion was noted. In the β 3 group at P70, the IFS of two thirds of the animals (3 of 5) remained completely patent, and the structure of the patent sutures was highly complex, closely resembling the structure of the patent SGS at P70.

Within IFS, the amount of bony bridging at P24 and the percent fusion at P70 in the β 3 group were significantly lower than in the other three groups as hypothesized ($P < 0.05$ at

P24, $P < 0.001$ at P70). The percentage of bony bridging or fusion within IFS was comparable in three other groups at both ages examined.

Changes in bite force with age

The changes in bite forces were similar to those reported previously (Shibazaki et al., 2007). Bite force increased with age, and a significant increase in bite force was found between P9 and P24 in all groups (Fig. 4; Table 2). While bite force in P24 and P70 rats with Tgf- β 3-treated IFS were lower than control and sham-operated groups, these differences were not significant.

Suture/Bone strains during incisor biting

The magnitude and orientation of maximum strain in the IFS were not significantly different between P9 and P24, were tensile in nature and were parallel to the IFS (Fig. 5A). However, a compressive force occurred perpendicular to the IFS at P70, and a significantly large compressive force was found parallel to the IFS at the same age (Fig. 6). Those changes were found in the CON, Sham, and Gel only groups. In contrast, tensile strain perpendicular to the IFS and less compressive strain parallel to the IFS were found in the β 3 group at P70.

The orientation of maximum strain was not significantly different between P9 and P70 in the SGS, but the magnitude of strain at P70 was significantly higher than at P9 (Figs. 5B, 6; Table 3). Although the magnitude of tensile strain was not significantly different between P9 and P24, the orientation of strain at P24 was nearly perpendicular to that at P9. This change in orientation of strain was present in all groups.

Compared to both SGS and IFS, strains in the PB were low in all groups. The orientation of maximum strain at P9 and P70 were similar to those seen in the SGS (Fig. 5C). The same strain patterns in the PB at P9, P24 and P70 were found in all groups (Fig. 6).

DISCUSSION

In the rat, all of the cranial sutures remain patent throughout life except for the posterior part of the IFS (Moss, 1954). This region of the suture undergoes osseous obliteration early, and the IFS fuses by P21 (Moss, 1957). Roth et al. showed that IFS from P12 – P20 were found to be actively fusing, and by P30, this suture was completely fused, with an occasional remnant of suture connective tissue visible at the periosteal (ectocranial) surface (Roth et al., 1997). All other cranial sutures remained patent. Previous research showed that Tgf- β 3 delayed IFS closure in rats, at least until P24 (Opperman et al., 2002), and that Tgf- β 3-treatment of a rabbit model of delayed onset suture fusion, allowed continued bone growth up to 84 days (Chong et al., 2003). This study showed a complex but patent IFS morphology in Tgf- β 3 treated rat IFS by P70, indicating that Tgf- β 3 treatment not only delays, but also inhibits normal IFS fusion for extended periods of time, possibly by preventing the loss of the suture mesenchyme as described by Holmes et al. (2009).

As was previously shown, the increases in bite force between P9 and P24 likely reflect changes from a soft to hard diet during weaning (Shibazaki et al., 2007). Bite force increased dramatically between P24 and P70 in all groups (Table 2), and no differences in bite force were noted between animals treated with Tgf- β 3 and control animals. The latter finding suggests that suture patency may be unrelated to bite force. However, the use of super-maximal pulses may have masked subtle changes in bite force related to changes in suture patency.

Strains parallel to the IFS were tensile at P9 and P24, and changed from tensile to compressive between P24 and P70. At P24 the Tgf- β 3 IFS showed less bony bridging than

controls, and the value and orientation of strain across the IFS were similar to the control groups. Since bite forces were similar between groups; this implies that strain levels were unchanged by delaying or inhibiting suture fusion. However, by P70 the Tgf- β 3 treated group continued to show less bony bridging within sutures than other groups, but now the tensile forces were perpendicular to the IFS. These latter findings are similar to those of Sun et al. (2004) who found that strain patterns in the posterior IFS that remained patent changed in strain pattern from pure compression to both compression and tension with increasing age, in contrast to the unchanged strain pattern in sagittal sutures (interparietal sutures) as they fused with age (Sun et al., 2004). These findings suggest that the continued presence of patent sutures can affect strain patterns when higher bite forces are present as in adult animals.

The SGS is situated between the parietal bones, and applying bite force resulted in a tensile strain perpendicular to the SGS. However, this angle changed from positive to negative at P24, and a tensile force now occurred parallel to the SGS. These changes occurred in all groups including P70, regardless of Tgf- β 3 treatment and changes in IFS suture patency. These data suggest that IFS patency does not affect SGS function, such that the IFS and SGS may work separately, and their biomechanics do not interfere or interact with each other.

Correlations between suture fusion and the global mechanics of the calvaria have been little studied, except in *in silico* simulations (Wang et al., 2010). In results presented here, P70 Tgf- β 3 treated IFS were patent or had less bony bridging than control sutures, and the biting forces and suture/bone strain were the same as in control rats. It appears then that artificial inhibition of IFS fusion does not have an effect on masticatory function. The suture morphology of the unfused P70 IFS was highly complex, winding through the frontal bone, similar to that seen in the SGS. Understanding whether the biomechanical effects of masticatory forces affect the morphology and patency of IFS and SGS needs more longitudinal studies. In the cranium, the dura mater has been believed to regulate significant suture growth events such as fusion or maintenance of patency (Opperman et al., 1993, 1995, 1998), yet it is unclear what regulates facial sutures. In rabbits, three facial sutures, the pre-maxilla-maxillary suture, the frontonasal suture, and the zygomaticotemporal suture and their immediately adjacent sutural mineralization fronts have markedly different suture complexity, and display different elastic properties and different capacities for mechanical deformation (Mao, 2002,2003; Mao et al., 2003a,b; Radhakrishnan and Mao, 2004). The question of whether facial sutures are more directed by extrinsic mechanical forces than calvarial sutures awaits further study (Wang et al., 2012).

It is intriguing that in many reptile and mammalian species the same sutures remain patent or fuse, regardless of craniofacial shape or dimensions (Krogman, 1930; Chopra, 1957; Herring, 1993; Hershkovitz et al, 1997; Rubidge and Sidor, 2001; Sidor, 2001; Wang et al., 2006b). It seems reasonable then to pose the question as to which comes first – fusion of the IFS, or a change of muscle mechanics with age? With this IFS delayed/inhibited suture fusion model, it is suggested that suture fusion affects the biomechanics of the craniofacial skeleton rather than *vice versa*. Thus, additional work is necessary to understand whether biomechanics of the craniofacial skeleton can have reciprocal effects on suture morphology and fusion.

The studies of suture biology and biomechanics call for more careful longitudinal experimental investigation. It is worth noting that the diversity of sutural morphologies (in both surface and cross-sectional views) poses a challenge to the analysis of sutural biology and biomechanics (Herring, 1993; Herring and Mucci, 1991; Rafferty and Herring, 1999; Byron et al., 2004; Markey et al., 2006; Markey and Marshall, 2007; Byron, 2009; Wang et al., 2012). In patent sutures, suture complexity (ectocranial sinuosity) and tissue

structure changes over time and with a change in masticatory forces (Massler and Schour, 1951; Gross, 1961; Milch, 1966; Kokich, 1976; Miroue and Rosenberg, 1975; Saito et al., 2002; Mao, 2003; Byron et al., 2004; Wang et al., 2006b; Byron, 2009; Smith et al., 2010). Other studies demonstrated that the suture interdigitation pattern is complex and diversified, and often disconnected from the ectocranial sinuosity pattern (Markey and Marshall, 2007). These authors demonstrate well that studies of suture morphologies and their interaction with their biomechanical environments call for the use of advanced imaging techniques, such as micro-CT or high resolution radiographs to accurately reconstruct the suture morphologies in a three dimensional environment.

Multiple biologic factors may play a role in the development, morphology, and biomechanical characteristics of sutures. It is interesting that Tgf- β 3-induced IFS suture patency produces suture complexity similar to the SGS suture. These findings indicate the complex interplay of growth factors and other molecular factors, biomechanical stimuli and suture patency in the craniofacial complex. This complexity calls for more coherent studies including various related fields such as fractal analysis (Yu et al., 2003), advanced imaging analysis and histological study (Markey et al., 2006; Markey and Marshall, 2007; Smith et al., 2010), *in vivo* and *in vitro* experiments (Herring and Mucci, 1991; Rafferty et al., 2003; Lieberman et al., 2004; Sun et al., 2004; Markey et al., 2006; Wang et al., 2008), investigation of the maturation of the craniofaciodental complex (Wang et al., 2006b, 2007; Wang, 2012), phylogenetic studies (Rubidge and Sidor, 2001; Sidor, 2001; Wang et al., 2006a), and computer aided simulations such as Finite Element Analysis (Kupczik et al., 2007, 2009; Farke et al., 2008; Moazen et al., 2009; Jasinowski et al., 2010a,b; Wang et al., 2010, 2012).

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FIG. 1.

A, Diagram showing a coronal section through the calvaria with a partially endocranially fused interfrontal suture (IFS) between left and right frontal bones. The thickness of the bones was measured, and the distance between top and bottom horizontal line gives the total width of the suture. Sutures begin fusing from the endocranial surface, so the distance between the middle and bottom horizontal lines gives the width of the fused region of the suture. This scoring method assessed the degree of sutural fusion across the thickness of the bones to test the effect of Tgf- β 3 or lack of it. This measurement was not meant to correlate suture morphology (such as interdigitation, beveling, and fusion) and biomechanics, which requires a three dimensional image database. B, Graph showing the percent fusion of sutures in each group.

FIG. 2.

Diagram of a rat skull showing placement of stacked delta rosette gages at two sutural sites and on one bone surface. IFS, Interfrontal suture; SGS, sagittal suture; PB, parietal bone.

FIG. 3.

Photomicrographs of histological sections through the posterior IFS of 24 and 70 day old animals. The ectocranial (periosteal) surface is superior and endocranial (dural) surface is inferior in all micrographs. Frontal bones are to the left and right of the suture (arrows). A, IFS of 24-day animals, showing sutures (arrows) in the control (con), sham operated (sham), and Gel only groups with bony bridging on their dural aspect (asterisks) by 24 days. In all cases the sutures fused from the dura side. Sutures in the Tgf-β3 treated group remained open (arrows). B, IFS of 70-day animals, showing gradual thickening of the bones compared to the 24-day animals. Note the progressive bony bridging (asterisks) across the 70-day control, sham and gel only sutures (arrows), with the persistent patency of the Tgf-β3 treated IFS (arrows). C, Low power composite images of sections through calvariae of control and Tgf-β3 treated animals. Note the thicker bones in the Tgf-β3 treated calvaria, with the highly complex IFS (arrows) winding through the bone.

FIG. 4.

Graph showing changes in bite forces with age in all treatment groups. Change in bite force with age was significant in all groups. No significant differences in bite force were noted between groups at any age.

FIG. 5.

A, IFS. B, SGS. C, PB. Graphs showing magnitudes and orientations of strain at three ages in four animal groups. Mean of three strain values, the maximum strain (tension), minimum strain (compression), and the strain channel (Channel 2; Ch2) or central element of rosette gage are plotted in age groups. The orientation of maximum strain was illustrated by rose distribution generated using the Oriana program for angular statistics. The central bold line in each circle represents the mean orientation, the brackets and two thin long lines linking their ends represent the circular standard deviations. Other thin lines represent individual orientations and sample size. On the graphs below the circles, means $\pm$ standard errors of the mean are given. At all ages and sites and in all animal groups, the change in strain patterns is remarkable between age 24 days and 70 days. The changes in orientation of the maximum stiffness are also significant.

FIG. 6.

Summary of strain patterns at the three gage sites, for the three ages and four experimental groups. Red lines with open arrows – maximum or tensile strain; blue lines with closed arrows – minimum or compressive strains. The lengths of the arrows are scaled to the mean magnitude, and their orientations are the mean orientations.

Table 1

Numbers of animals used in experiments

	CON (n=45)			SHAM (n=45)			GEL ONLY (n=45)			β 3 (n=45)			Sum
	Histo	Bite force & Strain		Histo	Bite force & Strain		Histo	Bite force & Strain		Histo	Bite force & Strain		
Day 9	5	10		5	10		5	10		5	10		60
Day 24	5	10		5	10		5	10		5	10		60
Day 70	5	10		5	10		5	10		5	10		60
Total	15	30		15	30		15	30		15	30		180

Table 2Biting force (mean $\pm$ SD)

	IFS measurement group	SGS measurement group	PB measurement group	Average
Day 9				
Con	55 $\pm$ 22	79 $\pm$ 15	84 $\pm$ 13	73 $\pm$ 20
Day 24				
Con	803 $\pm$ 118	690 $\pm$ 167	630 $\pm$ 141	707 $\pm$ 150
Sham	677 $\pm$ 134	630 $\pm$ 163	695 $\pm$ 80	667 $\pm$ 124
Gel only	727 $\pm$ 144	604 $\pm$ 151	610 $\pm$ 169	647 $\pm$ 155
β 3	566 $\pm$ 139	458 $\pm$ 311	710 $\pm$ 57	578 $\pm$ 213
Day 70				
Con	2451 $\pm$ 308	2585 $\pm$ 229	2241 $\pm$ 105	2426 $\pm$ 255
Sham	2108 $\pm$ 550	2159 $\pm$ 574	1833 $\pm$ 381	2033 $\pm$ 494
Gel only	1766 $\pm$ 426	1783 $\pm$ 676	2160 $\pm$ 721	1903 $\pm$ 606
β 3	1840 $\pm$ 709	1732 $\pm$ 429	2049 $\pm$ 549	1874 $\pm$ 548

 $P < 0.05$ among treatments. Unit: gf (1 gf = 9.80665 millinewtons)

Table 3

Peak strain and orientation (mean±SD)

Rat age, group	Interfrontal suture (IFS)				Sagittal suture (SGS)				Parietal bone (PB)			
	Ch 2 (μe)	max (μe)	min (μe)	α (°)	Ch 2 (μe)	max (μe)	min (μe)	α (°)	Ch 2 (μe)	max (μe)	min (μe)	α (°)
Day 9												
Con	-118±74	224±128	-156±50	173±12	-105±64	177±99	-70±80	89±4	91±34	101±51	-28±66	94±11
Day 24												
Con	-90±23	154±80	-96±43	171±14	-124±110	175±98	-165±114	7±5	17±24	58±10	-18±30	138±7
Sham	-159±111	192±74	-236±126	10±20	-102±45	152±58	-117±45	14±4	40±20	59±22	-30±15	119±6
Gel only	-57±50	141±74	-110±102	11±21	31±53	107±45	-26±48	179±19	38±66	71±26	-44±44	108±29
β3	-190±67	179±154	-257±131	172±6	-29±16	210±97	-132±92	15±30	-46±66	65±63	-85±18	3±34
Day 70												
Con	-128±55	-78±33	-408±237	106±7	892±485	937±477	-202±145	96±11	125±39	139±38	-15±32	101±16
Sham	-159±79	-114±94	-349±78	126±13	628±415	701±395	-229±139	104±13	103±36	122±36	1±34	102±29
Gel only	-66±153	-31±135	-419±110	106±9	528±328	561±311	-211±114	91±14	131±28	166±10	-33±31	113±11
β3	-57±92	118±122	-257±131	95±20	559±339	602±326	-205±156	92±14	234±100	239±98	-81±75	93±6

* Ch 2: the rosette gage element perpendicular to sutures, max; maximum strain, min; minimum strain, α; orientation of maximum strain . $P<0.05$ among treatments.