

SM Alaqeel
RJ Hinton
LA Opperman

Cellular response to force application at craniofacial sutures

Authors' affiliations:

Samer M. Alaqeel, Robert J. Hinton, Lynne A. Opperman, Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, TX, USA

Correspondence to:

Lynne A. Opperman PhD
Department of Biomedical Sciences
Baylor College of Dentistry
Texas A&M University System Health Science Center
PO Box 660677, Dallas, TX 75266-0677, USA
Tel.: +1 214-828-8134
Fax: +1 214-828-8951
E-mail: lopperman@bcd.tamhsc.edu

Structured Abstract

Authors – Alaqeel SM, Hinton RJ, Opperman LA

Objectives – To provide a comprehensive review of the literature describing research done on the responses of suture cells to force application *in vitro* and *in vivo*.

Design and Results – This review outlines the types of forces that can be applied, methods of applying the forces, the sutures used in experiments, and the changes in morphology, molecular biology (gene and protein expression), and cell biology (proliferation, differentiation, apoptosis) in response to these forces.

Conclusion – The molecular response of sutures to force needs to be further investigated as these molecules can be used to enhance the way in which craniofacial sutures respond to mechanical force during orthopedic–orthodontic treatment.

Key words: cranial sutures; growth and development; growth substances; mechanical stress; mechanotransduction; orthodontics; orthopedic procedures

Introduction

A relationship between mechanical stress and bone formation was suggested by Julius Wolff in the late 19th century (1). His suggestions, which are known as Wolff's law, state that bone remodels according to the mechanical demands that it has to withstand. When mechanical loads are high, bone forms and its structure remodels in ways that suit its function. The use of mechanical force to correct some dentofacial deformities was pioneered by Angle and Kingsley (2,3). Since then, it has widely been accepted that many

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craniofacial deformities can be corrected by the application of various sorts of mechanical forces. Orthodontists and orthopedic surgeons use force to grow bone at the bony margins of many sutures in the craniofacial complex, such as the midpalatal, inter-premaxillary, palatomaxillary, sagittal, and coronal sutures. With the application of mechanical stresses of different polarities, orthodontists and orthopedic surgeons were successfully able to alter the dimensions of a cranial bone by millimeters or sometimes centimeters (4). Craniofacial sutures are of interest in this review because during orthopedic treatment, most if not all of the bone growth that occurs in the craniofacial complex is attributed to anabolic activities at the sutural fronts. Sutures are considered as the growth sites of intramembranous bones in the craniofacial complex. Accordingly, it is fair to assume that if sutures were not present, craniofacial bones might grow only in thickness (5,6).

The tissues surrounding sutures, such as the dura mater, have a significant effect on sutural patency and growth (7–9). Patent sutures are not solid and have Young's moduli lower than that of their neighboring bones, and accordingly they respond to mechanical loads and function as a cushion between their adjacent bones. On the contrary, obliterated sutures have Young's moduli comparable with that of their neighboring bones, and as such they are insensitive to mechanical forces (10,11). Sutures are dynamic and respond to different types of mechanical stimuli. The application of force does not always result in anabolic cellular activities; it might result in catabolic activity depending on the polarity and magnitude of force. The application of tensile force usually results in sutural growth, whereas compressive force might result in cell death and sutural resorption (12–15).

The intention of this review is to delineate the different events that occur at the cellular and molecular level during the application of different types of forces on craniofacial sutures.

Types of force application at craniofacial sutures

High strains of as much as 1000–2000 micro-strain were found *in vivo* in sutures such as the zygomatic suture, which are relatively larger than those detected

in their neighboring bones (16). Higher strains are an indication of lower Young's modulus, which indicates that sutures are easier to deform than their respective bones.

The polarity of stress can change dramatically depending on the location and magnitude of force (16–20). The application of force on some sutures, such as the zygomaticotemporal suture, produced contrasting cellular effects. Some parts of the suture responded with bone resorption, whereas other parts responded with bone formation. This was due to the complex types of stresses that resulted from the application of force. As a result of the interdigitation of sutures and their three-dimensional complexity, the application of force to one area of a suture causes different complex stresses in different areas of the same suture (21). When these parts were studied in dry skulls, it was elucidated that not all parts of the same suture experienced the same form of force. In general, it was found that the medial part of sutures experienced compressive stresses, while the lateral part experienced tensile stresses (22). Accordingly, it is very important to distinguish stresses with different polarities and recognize which force would result in what kind of cellular response (10,23). Complex forces not only expand or contract the suture, but also produce complex movements in the suture that might result in a complete displacement or movement of the suture (21).

During normal physiological conditions, craniofacial sutures are subjected to one or more types of stress. The first type of stress results from the growth of the brain, which pushes the bones of the cranial vault in an outward direction. This type of stress is unique, in that it causes compressive stress on the internal surface of the cranial vault and its sutures, while the external surface of the vault and the sutures encounter tensile stress, which usually causes growth of the suture and the bony vault (10,24). The other type of stress results from function of the masticatory muscles. The force generated by the muscles is usually transmitted to the neighboring bones and sutures, and it can produce both tensile and compressive stresses (17,19).

Tensile and compressive stresses are not the only types of stress experienced by craniofacial sutures, as shear stress has also been reported to occur (25–27). Unlike compressive and tensile stresses, the detection of shear stress is much more complicated and requires advanced analysis techniques. Compressive and tensile

stresses can be measured directly by placing strain gages across sutures. However, the measurement of shear stress requires the placement of multiple sophisticated strain gages in more than one location at the suture, which makes it almost impossible to accurately detect stresses. New analysis techniques such as finite element analysis (FEM) elucidated that high levels of shear stress can be found at the concave surfaces of craniofacial sutures when the suture is under tension (27). The conversion of tensile and compressive stresses to shear stress can be attributed to the presence of the fibrous connective tissues that occupy the sutural space and connect the sutural bony fronts.

Different orthopedic devices are capable of applying different types of exogenous forces, and the basic difference between them is the direction in which the force is applied to the suture. Simply, when the force is directed outward from the suture, the force will be a tensile one. On the contrary, if the force radiates from the sides of the suture toward its center, then the force will be a compressive one. Shear stresses are not usually applied directly to the suture, but they are rather a result of complex array of forces that result from two components of stress acting at directions opposite or oblique to each other (Fig. 1) (16,19,20,22). As shear stress might arise during the application of tensile or compressive stresses, it should be noted that a component of shear stress might contribute to the results observed during the course of sutural stress experiments, which might accordingly affect the accuracy and reliability of the outcome (28,29). This is especially true if the cellular response to force application is the

aim of the study, as the effect of shear stress might be contrasting to that of the tensile stress and analogous to that of the compressive stress (27). Unfortunately, the determination of the exact level of the shear stress component that results during tensile or compressive stress research is a difficult or maybe an impossible task by the current available means, and more attention needs to be paid to the role of shear forces in future suture biomechanics studies.

Methods of force application on craniofacial sutures

Both *in vivo* and *in vitro* methods have been used to study forces in craniofacial sutures. The importance of establishing a well-controlled *in vitro* model was first recognized by Meikle and his coworkers (30). They developed a practical experimental model, in which cranial sutures can be observed and studied under the influence of a controlled mechanical force. They mounted sutural explants on split circular mounts, which were capable of applying a controllable level of tensile stress on the suture. The force magnitude that would be delivered to the suture, using this method, was adjusted by changing the gap between the two halves of the circular mount. The thickness and the number of coils of the wire, which was wedged between the two halves of the mount, also assisted in controlling the magnitude of the force delivered to the suture (Fig. 2). The circular-mount method could also be used for applying compressive stresses. This was done by wrapping a helical metal spring or a rubber band of known force around the circumference of the cylinder. The advantage of using the circular-mount method, which was adopted by many researchers (30–32), was that its perforated surface provided some sort of retention to the surface of the suture, and accordingly, the applied force was distributed equally to the entire area of the suture (30,32).

Another popular *in vitro* method was based on the placement of helical springs in holes drilled laterally to the center of the suture (33–36). This latter method was also adopted in many *in vivo* studies, in which the study subjects underwent surgical placement of helical springs in pre-drilled holes lateral to their suture of interest (12,13,37–41). Not all *in vivo* suture studies were performed by direct force application to a suture.

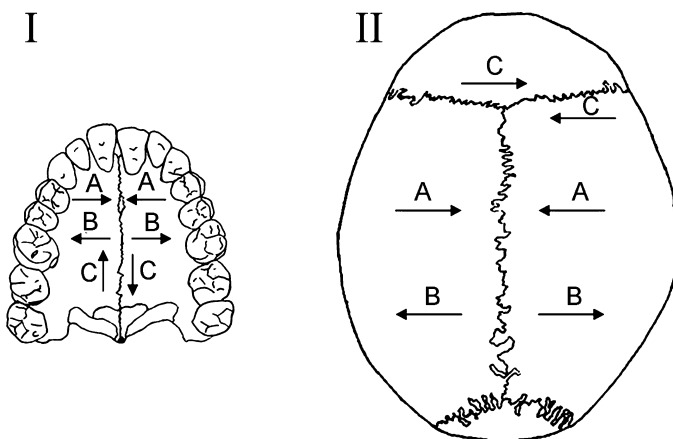


Fig. 1. Different stress types acting on different sutures. I. The intermaxillary suture, II. The coronal suture and the sagittal suture. (A) Compressive stress; (B) tensile stress; (C) denotes shear stress.

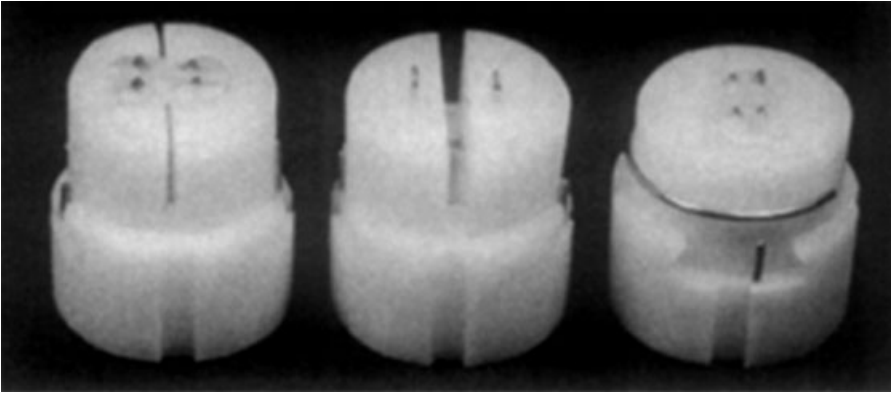


Fig. 2. Split circular mounts used to apply mechanical stress on sutures. Adopted from Meikle *et al.* 1979 (30).

In some cases, an indirect force application was accomplished by placing a mechanical device or a spring on the teeth surrounding a suture (23,42–44), or by altering the force transmitted to a suture via a functional muscle, either by dissecting the muscle, by changing the length or mass of the muscle, or by changing the diet consistency of the subject (25,45,46). Previously mentioned methods are not very accurate as they are vulnerable to inaccuracy in maintaining the magnitude of the applied force. The change of the magnitude of force was due to the change in the mobilization resistance of the bone and the change in the distance between the two bone fronts at the sides of the suture (44). Using these methods, the spring or the elastic device applied higher magnitudes of force at the beginning of the experiment, and as the suture increased in width the device applied a lower magnitude of force. More accurate control of the applied force was accomplished by using devices that are capable of applying the same magnitude and rate of force regardless of the distance change due to the expansion or the contraction of the suture. In such studies, a force was applied using floating pistons or mechanical loading machines (47,48).

Sutures used in force studies

Almost all accessible craniofacial sutures have been used in force studies. The rationale for choosing a specific suture for conducting a study is mostly dependent on the aim of the study. Accessibility is one of the most important factors that is considered before conducting a study. Because most of the tested craniofacial sutures responded the same to the application of force, researchers used the sutures that were the

easiest to access. Sagittal and coronal sutures were the most often tested sutures in response to force application *in vivo* (25,40,49). This is due to the fact that these sutures can be accessed easily with the least discomfort to the animal. The soft tissues covering these sutures have very low blood supply and are devoid of muscle attachments. The effect of force on sutures has been intensely studied in the maxilla, as the maxillary sutures are the key targets during the treatment of a narrow maxillary arch. As the components of the craniofacial complex, which include sutures and bones, interact as a dynamic model, sutures close to the maxilla, such as the zygomaticomaxillary (21,35,50,51) and the frontonasal suture (52), were also studied. The maxilla has three major sutures that have been studied intensely: the intermaxillary (midpalatal) suture (35,43,45,46,50,53–59), the premaxillomaxillary suture (42,60), and the palatomaxillary suture (60), to determine the effect of the application of load, as in maxillary lengthening or widening, or even maxillary retraction (61).

The response of sutures to stress application

It is not well understood if the craniofacial skeleton responds to mechanical stress in a similar fashion as the appendicular skeleton. There are many differences between the craniofacial and appendicular skeleton, as the former is derived from neural crest cells, while the latter is generally derived from mesoderm. Growth plates in the appendicular skeleton are generally resistant to external forces. However, sutures are known to respond to epigenetic factors (62,63), such as mechanical stresses.

Mechanical stress causes deformation in elastic objects, of which sutures are a good example. It has been suggested that stress-induced physical changes in the plasma membrane of connective tissue cells within the deforming suture matrix initiate a cascade of signaling events within the cell (64–66). When connective tissue cells under stress were examined (36), cell membrane permeability was noticeably higher than in unstressed sutures. Intracellular Ca^{++} increased rapidly and, consequently, caused an immediate cellular response to the application of tensile stress. This response caused increased cellular activity at the suture, and accordingly, affected the growth and morphology of the suture (36). As mentioned elsewhere in this review, different polarities of mechanical stimuli might produce different types of responses: e.g. tensile strain might cause anabolic bone activity while compression might cause catabolic changes and osteoblastic apoptosis (12–15,67). Accordingly, the effect of each type of stress will be examined separately hereafter.

Changes in morphology, structure, and interdigitation of sutures in response to stress

Sutures play the role of shock absorbers, in which they dissipate stresses from their adjacent cranial and facial bones during impacts on the craniofacial complex. The energy, which the suture dissipates or absorbs during impact, is positively correlated with the suture's level of interdigitation (68). Interdigitation helps in providing a larger surface area at the bone interface for collagen fibers to attach to the bone fronts and accordingly helps in alleviating the stress (68,69). Sutures that resist compression have more complex morphology and interdigitation at the interface when compared with those that resist tension (16).

Morphology and structure change when mechanical force is applied to craniofacial sutures, such as the interparietal suture (38,70). Tensile force causes sutural width to increase significantly in the lateral direction, directly proportional to the magnitude of the applied force (38,70). Vardimon *et al.* (59) reported a 12-fold increase in the radiolucent area of the suture; however, during the retention stage, sutures narrowed to 9-fold greater than the original width, and mineralization increased significantly. While the application of tensile force at sutures increased bone formation, it prevented total mineralization of the sutural bone fronts, and the

mineralization of bone increased dramatically after force application ceased (59). The application of tensile stress at palatal sutures also caused the lateral cartilaginous layers to move farther from each other and to decrease in width as they were replaced by bone (44,71). Alteration of sutural morphology is accompanied by bone remodeling activity, as evidenced by the presence of resting and reversal lines and cement lines (21,52).

In one study it was reported that the application of force along with irradiation might alter sutural morphology and structure (40). This experiment showed an increase in the distance between two holes created lateral to the suture. In addition, there was a noticeable extension of the transverse fibers at the suture, and significant enlargement of the blood vessels in the vicinity of the suture. It was concluded from this study that the suture responded to treatment, but it was unclear if the response was due to the expansive force, irradiation, inflammatory reaction, or all of these factors, as the control group was not exposed to any of these factors. Myostatin-deficient mice, which have significantly higher muscle mass than wild-type mice, exhibited greater bone formation at the sutural fronts than that occurring in the wild-type mice. This may be attributed to the higher bite force found in the knockout mice. A surprising finding in this study was that during the mechanical separation test, sutures with higher interdigitation had lower stiffness than those in the wild-type mice. This finding might be attributed to the rapid formation of connective tissues around the suture, which would result in more flexibility. However, even though the sutures were stiffer in the knockout mice, they did not show higher breaking strength (25).

Compressive stress was also reported to cause morphological and structural alterations in the suture. The width of the suture increased significantly and large numbers of Howship's lacunae were found on the bony borders of the suture. Numbers of blood vessels and bone deposition increased in all areas of the suture. These changes were permanent, as there was no significant remodeling in the sutural vicinity after 2 months of observation; rather reorganization of the fibrous suture took place. Experimental sutures were significantly wider and more complex and irregular than controls (55). Sutures subjected to compressive forces formed abutting morphology and stopped growing, eventually becoming obliterated. Both

compressive and tensile stresses also changed the orientation of the sutural fibers, as it was found that obliquely oriented fibers are the best suited to resisting the mode of strain being applied to them. It has been shown that high compressive strain caused increased interdigitation at the suture, but no bone resorption; interdigitation did not increase in response to tensile stress (72). The effect of stress on sutural interdigitation was found to be related to the age of the animal as the interdigitation decreased with age until the sutures became smoother-edged (10).

Changes in protein levels and the ECM in sutures in response to stress

Although there is a consensus among researchers that tensile stress increases the protein level in the ECM, there were contrasting reports about the levels by which protein increased. By using protein markers such as ^3H -leucine, ^3H -proline, and ^3H -thymidine, researchers were able to identify which type of protein changed in response to application of force. It was suggested that the expansion of a suture by means of mechanical stress caused gradual suture widening followed by production of connective tissue, to restore the original morphology of the suture (12,70). Tensile force applied to sutural explants caused an increase in the metabolic activity which resulted in two- to threefold increases in the overall protein synthesis. This increase continued for several days following the application of tensile force (73). The increased protein was mostly in the ECM (30,32,37). It has been suggested that when there is a necessity to add more extracellular matrix at the edges of the suture, as in a suture under tension, the suture will respond by producing more collagen type III (37,49). It was reported that the synthesis of collagen type III increased in response to force application and peaked at the third experimental day; thereafter, it tapered off to its initial levels. It is noteworthy that after 6 h of tensile force application, collagen type III synthesis was higher following low-magnitude forces than high-magnitude forces. However, there was no noticeable sutural expansion at 6 h in the low-magnitude force group. This suggested that low-magnitude forces may initiate the remodeling process more efficiently than high-magnitude forces (38). In another study, it was reported that protein synthesis and increased sutural width were more sen-

sitive to force duration than to force magnitude (39). Moreover, the effect of force was more pronounced on the ectocranial than the endocranial surfaces of the suture (39).

Changes in TIMP and in MMP expression in sutures in response to stress

Collagens are the major structural units of connective tissues. Connective tissue stability, function, and resistance to nonspecific degradation by proteolytic enzymes are due to the structure and support of the collagens. The resistance to degradation of collagens is mainly due to molecules such as proteoglycan, elastin, and fibronectin, which help protect the sensitive parts of the collagen from any proteolytic activities. Matrix metalloproteinases (MMPs), which are secreted from connective tissue cells, can break down many structural macromolecules in the connective tissue matrix. To prevent their destructive actions, MMPs are secreted in an inactive form and must be activated in order to function. Connective tissue cells also synthesize inhibitors of these destructive enzymes called tissue inhibitors of metalloproteinases (TIMPs). There exists a balance between the two antagonist molecules to keep the connective tissue matrix in a stable state (74–76).

In sutures, the application of tensile force for 48 h increased the level of proline incorporation into the sutural tissues; however, the total protein content of sutures did not increase (13). This indicated that changes in protein synthesis and protein degradation were occurring simultaneously. MMP levels initially increased in stressed sutures and then declined. However, increased MMPs in sutures did not affect the degradation rate of collagen molecules, a fact attributed to a small increase in TIMP expression (73). Collagenase, gelatinase, and neutral metalloproteinases were abundant around the densely packed cells in the center of the suture and at the periosteal surface; however, fibrous tissues were negative (32). TIMPs were found inside the cells of the central suture area, but not in the area of increased cell proliferation (31). These experiments showed that the tensile mechanical stress stimulated the production of proteins as well as the enzymes that are responsible for their hydrolysis. Interestingly, the inhibitor of those enzymes also increased in response to mechanical stress, suggesting that TIMPs were produced to counteract the function

of the MMPs and to protect the matrix from complete degradation (32).

Changes in proliferation and differentiation of suture cells in response to stress

Changes in sutural width in response to stress were closely correlated with cell number and cell activity. Sutures narrowed or widened depending on the type of stress acting upon them; however, even when sutures increased in width some destructive cells, such as osteoclasts, increased in number (33). The final ratio between osteoblasts and osteoclasts will determine if the suture is in a resorptive or productive phase of bone turnover. Changes in the metabolic activity of sutural cells were reported in mechanically stressed suture (33,73). Levels of cell proliferation and collagen fiber synthesis in the cranial suture were positively correlated with the level of the tensile stress (13,33). The application of tensile stress, even at low magnitude, caused a gradual increase in the number of osteoblasts, osteocytes, and blood capillaries. In addition, tension caused a noticeable decrease in the number of osteoclasts (14,34,48,52). The number of fibroblasts was also very sensitive to the force magnitude, as the numbers of fibroblasts increased rapidly to a very high level in response to increased force magnitude.

As sutures are composed of fibrous tissues that are more elastic than their surrounding bones, any mechanical force applied to the surface of the cranial vault will be transformed into tensile stress at the bone fronts around the suture (77). When the force source is a metal spring mounted on the ectocranial surface of the skull, the effect of force on sutural growth was more pronounced at the ectocranial surface than the endocranial surface of the suture. This difference in bone growth may be attributed to differences in the magnitude of strain between the ectocranial and endocranial surfaces, as the level of tensile stress is always higher at the outer surface of any curved plane than at the inner surface (10,39).

It has been postulated that even in the absence of exogenous applied stresses on craniofacial sutures, the sutures will still experience considerable amounts of tensile stress. The presence of tensile stress at the sutural bone fronts is a result of two mechanisms: the growth of the brain and the stress transmitted from functional muscles (63). The growth of the brain causes

tensile stress on the suture in two ways: the separation force resulted from the expansion of the brain (24,77), and the remodeling of the ectocranial and endocranial surfaces of the cranial vault (25). Pressure on the endosteum from the brain causes the inner surface of the vault to experience compressive stresses and the outer surface to experience tensile stresses. Accordingly, bone resorption occurs at the inner surface and bone deposition at the outer surface of the vault, which subsequently cause an increase in the volume of the cranial vault. Even though the process of remodeling is slow, it causes tension to occur at the bone fronts, which triggers a compensatory response at the sutures. Functional muscles that are acting on the craniofacial complex also cause tension to occur at the craniofacial sutures, which accordingly respond with bone deposition (19,24).

Osteoprogenitor cell numbers in sutures increased in response to tensile stress application, and their increase was proportional to both the duration and magnitude of applied force. However, the increase in number was more sensitive to the duration of stress than it was to the stress magnitude. The number of osteoprogenitor cells increased in response to high-magnitude tensile stress at the beginning of stress application, and then decreased rapidly. Furthermore, osteoprogenitor cell number was equal to that in sutures exposed to low stress levels but for longer duration (23,42). The application of compressive stress to sutures caused a contrasting behavior. The number of osteoclasts increased significantly, and osteoclastic activity exceeded that of osteoblasts, which accordingly caused a net decrease in the sutural width (55).

The application of tensile forces on the midpalatal suture caused a change in the differentiation pathway of osteo-chondro-progenitor cells. This was inferred from the observed width decrease in the lateral cartilaginous layer, which was then replaced by bone, and the width increase in the precartilaginous layer, which was displaced laterally by fibrous tissues at the center of the suture (71). Tensile stress applied to cranial sutures also induced osteoblast differentiation and, accordingly, bone formation (35). Compression of sutures also induced cell differentiation and increased the levels of type I, type II, and type X collagen expression in the precartilaginous and cartilaginous cell layers of some craniofacial sutures (78).

The effect of shear stress on cell proliferation has not been widely studied. This is due to the difficulty of determining the exact magnitudes and locations of the shear stresses at a suture. It has been reported that shear stress was found on concave surfaces during the application of tensile stress on sutures (27). This observation might contribute to the explanation of the resorptive activity found on concave sutural surfaces where osteoclasts concentrate and localize to perform their activity (79).

Changes in growth factor expression in sutures in response to stress

Like other craniofacial tissues, craniofacial sutures contain several growth factors, including Bmp-2, Bmp-4, Igf-I, Fgf-2, and the isoforms of Tgf- β : Tgf- β 1, Tgf- β 2, and Tgf- β 3 (62,81,82). There is some evidence that their presence in sutures might be regulated by mechanical stimuli.

It was reported that the production of Igf-I and Igf-IR mRNA in sutures increased in response to the application of tensile force (34). Furthermore, the number of osteoblast-like cells and fibroblast-like cells increased dramatically in response to the applied force. The increased Igf-I and its receptor led to the increased proliferation of osteoblast-like cells and fibroblast-like cells noted in the mid-sagittal sutures under tensile stress (34). The presence of Igf-I along with sufficient force stimuli encouraged bone remodeling and growth because of higher osteoblastic and osteoclastic cell activities. Even though osteoblast and osteoclast cell counts increased in response to Igf-I injection and mechanical stimulation, osteoblast cell number was much greater than the number of osteoclasts. Therefore, it is clear that Igf-I and mechanical stimulation have an anabolic effect on sutural tissue, in which they boosted bone formation more than bone resorption (80).

Tgf- β 1 is an important factor for the formation of bone. Sawada and Shimizu reported that the expression of Tgf- β 1 by sutural osteocytes and fibroblasts was elevated (43), especially toward the beginning of stress application. Thereafter, Tgf- β 1 levels quickly tapered off until they reached Tgf- β 1 levels found in relaxed sutures. Stressed sutures were devoid of tartrate-resistant acidic phosphatase (TRAP), which is a marker for osteoclasts (43). When the effect of exogenous Tgf- β 1 was evaluated with respect to bone formation during

force application, it was found that injecting animals with a single dose of 200 ng resulted in similar outcomes as injecting animals multiple times at different points during force application. The effect of Tgf- β 1 was dose dependent only if the dose was below 200 ng. When animals were injected with doses <200 ng, bone formation was less than in animals injected with \geq 200 ng. Therefore, exogenous Tgf- β 1 may play an important role in enhancing the production of bone during rapid sutural expansion (43).

Fgf-2 was found to rapidly increase 150% in stressed sutures when compared with non-stressed ones. This increase resulted in conjunction with a noticeable increase in the cell membrane permeability, and increased intracellular Ca⁺⁺ (36). Increased sutural cellular proliferation and differentiation were also noted, which accordingly resulted in increased growth of the suture (36).

In a study that evaluated the effect of stress application on Bmp4 expression, it was found that Bmp4 was significantly upregulated in response to force application. Bmp4 upregulation was accompanied by Cbfa1/Osf-2 expression in preosteoblastic and fibroblastic cells. Increased stress also induced noticeable expression of Bmp4 in preosteoblastic and fibroblastic cells (35).

Growth factors function by transmitting their signals to their respective receptors, which are located on many functional cells. Accordingly, additional studies are needed to investigate the effect of mechanical stress on the regulation of the growth factors' receptors.

Changes in transcription factors expression in response to stress

Transcription factors such as Twist, Msx2, Runx2 (Cbfa1), and Tbx2 are known to play a role in sutural morphogenesis (62,81). Accordingly, it is important to look at their expression level during the application of load at craniofacial sutures to better understand the mechanisms by which the sutural morphology changes.

Few studies have examined the effect of mechanical stimuli on the regulation of transcription factors. It was found that the expression of Tbx2 increased two- to threefold after as little as 5 min of stretching the suture. At the same time, the expression of Cx43 decreased three- to fivefold. Western blot analysis confirmed an antagonistic relationship between the expression of

Tbx2 and Cx43, given that when Tbx2 was upregulated, CX43 was downregulated when the suture was under mechanical force application (47).

The effect of stress relief on craniofacial sutures

Mechanical stimuli cause sutural growth and remodeling, leading to the speculation that the absence of mechanical stimuli would have an adverse reaction on sutural tissues. The relief of stress can be induced by a variety of methods such as severing the muscles that act on sutures, paralyzing the muscles, or isolating the suture from the surrounding bones and muscles (82). Space flights might not be very effective in relieving craniofacial sutures from stress, because most of the forces that act on craniofacial sutures are induced by the muscles of mastication. Therefore, even though there would be little required work to move the jaw in space, force will still be applied from applying pressure on the opposing teeth. Some researchers used soft food to decrease the masticatory forces transmitted to sutures (46), but using this method will still allow some force to be applied to sutures. Accordingly, this method will not simulate some clinical situations in which complete immobilization of the suture takes place.

The compressive and tensile forces generated by masticatory muscles are invaluable to the development and maintenance of craniofacial sutures. The absence of the effect of these muscles altered the sutural morphology and made sutures less interdigitated. When complete muscle removal was done in animals at early developmental stages, some craniofacial structures were prevented from developing. With the isolation of the sagittal and coronal suture from their surrounding bones and muscles, it was clear that the sutural area lost its interdigitation and the sutures were smooth and non-beveled (82).

When sutural immobilization was performed on rabbits (83), bone bridging across the sutures occurred in a short period of time. However, this bridging was only at the ectocranial surface of the suture. Immobilized sutures showed minimal growth, even after as much as 90 days. When immobilized sutures were craniectomized from a few subjects in the study, a compensatory growth response occurred. The bone at the

sutures grew very rapidly and skulls reached the same levels of growth as their sham peers. Surprisingly, the removal of the sutures from few subjects in the sham group resulted in growth burst that exceeded normal control rabbits, and exceeded the growth level of the operated rabbits that had their sutures craniectomized. This observation contradicts researchers who stated that the skull will not grow bigger without the presence of sutures (11,62,63). However, the observed response might only be due to the inflammatory response that occurred because of the removal of the suture. This study showed that craniosynostosis can be artificially induced, and might be treated at an early age by the removal of the affected sutures. However, this paper lacked sufficient histological analysis and immunohistochemistry techniques that might help in elucidating what cell activity was in effect at the sutural area (83).

The response to sutural immobilization is dependent on the duration of the immobilization and on the age at which immobilization was performed (84). When very young animals were studied, they showed no sutural growth at the experimental site and showed decreased sutural irregularity in comparison to the control site. However, the suture remained patent at 30 and 60 days. In non-growing older animals there was no effect of the sutural immobilization. This might suggest that in older animals, even before the immobilization was performed, there were no more extraneous forces to cause bone growth at the suture, or simply the suture might already have obliterated.

When compressive stresses from the cheek muscles, which act bilaterally on the maxillary teeth were relieved, there was a striking increase in the intermaxillary molar span (45). Decreased mechanical force by decreasing the mastication force transmitted to sutures, such as the intermaxillary suture, caused a noticeable reduction in the level of DNA synthesis (46). The relief of stress also decreased the cartilage matrix at the suture, and increased the osteogenic activity, as most of the cartilage present at the suture was replaced by bone. The rate of these effects decreased as the duration of the experiment increased.

Ozaki and coworkers considered cranial synostosis as a form of stress relief at the sutural area (85). Accordingly, these researchers assumed that completely synostosed sutures do not experience any mechanical force, and patent and partially patent sutures encounter stresses that are proportional to their patency. μ CT

analysis showed that bone volume fraction and mean trabecular thickness were high in the open region of the suture and were lowest in completely synostosed sutures. Bone surface to bone volume ratio and mean trabecular separation were high in the complete synostotic sutures and lowest in the open region. Histological examination of open sutures showed that osteoid was present along the ectocranial surface and at the suture bone fronts, whereas in partially synostosed sutures osteoid was only found at the ectocranial surface and not at the bone fronts. As it is difficult to determine if the force is really different between different portions of the partially open suture, the effects reported might be attributed to factors other than those reported, such as the absence of growth and transcription factors that participate in maintaining the sutural patency (62,86–90).

Conclusion

In this review, the different events and different factors that might affect the response of craniofacial sutures to mechanical stimuli have been reported. Understanding the way by which craniofacial sutures respond to mechanical force is fundamental, as mechanical force plays an important role in the growth and development of craniofacial sutures. Orthopedic–orthodontic therapy and the treatment of many craniofacial deficiencies require, in most cases, a non-surgical modification of one or more craniofacial sutures. Many factors such as the amount, polarity, and duration of force application play an essential role in the success of such treatments. Craniofacial and developmental biologists researched sutures for they are considered as growth sites in the craniofacial region. Accordingly, the molecules, factors, and the interaction of sutures with their neighboring tissues were specifically researched to delineate the different events that take place during sutural modifications. Additional work is needed to elucidate the mechanotransduction events by which craniofacial sutures respond to the application of mechanical force. The regulation of growth and transcription factors and their receptors needs to be further investigated as these molecules, if well understood, can be used to enhance the way in which craniofacial sutures respond to mechanical force during any orthopedic–orthodontic treatment.

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