Sutural expansion using rigidly integrated endosseous implants: An experimental study in rabbits

J. Andrew Parr, BS; Lawrence P. Garetto, PhD; Mark E. Wohlford, PhD, DDS; Gordon R. Arbuckle, DDS; W. Eugene Roberts, PhD, DDS

Routine orthodontic/orthopedic therapy as well as correction of specific craniofacial skeletal anomalies (such as cleft palate) may require the expansion of one or more of the facial sutures. Traditionally, the teeth are used as anchors for applying loads to expand the sutures. The drawbacks of using dental abutments for orthopedic expansion include the generation of unwanted tooth movement,[1-4] root resorption,[5] and lack of firm anchorage to retain sutural expansion.[6] One method of avoiding unwanted tooth movement is the use of intentionally ankylosed teeth as abutments.[2-4,7] However, intentionally ankylosed teeth have a limited lifespan due to root resorption and eventual exfoliation.[1,3] In addition, they may not be in the ideal location for appropriate force application.[1] Furthermore, orthodontic movement of ankylosed teeth is not possible, which may limit occlusal correction. An additional limitation for use of teeth (ankylosed or not) as anchors for sutural expansion is that many patients with craniofacial anomalies have multiple congenitally missing teeth. In these cases, surgery or soft-tissue-supported appliances are often the only available treatment.

Abstract

Rigidly integrated implants offer great promise for orthodontic and orthopedic anchorage in the oral and midfacial regions. Rigid anchorage can be used to control unwanted tooth movement, provide abutments in edentulous arches, and open the vertical dimension of occlusion. To evaluate the use of endosseous implants in the midface region, two flanged titanium implants were placed on either side of the midnasal suture of 18 New Zealand White rabbits. The rabbits were divided into an unloaded control and two experimental groups. One experimental group was loaded at 1 Newton (N) and the other at 3 N. All rabbits were euthanized after 12 weeks of loading. Stereologic point-hit and line-intercept methods were used to analyze microradiographic and multiple fluorochrome histology of the suture. All implants remained stable during the loading period. The distance between the implants increased significantly in the loaded groups compared with the control, and was significantly higher in the 3 N group than in the 1 N group. Percent bone volume was significantly decreased, while the percent suture volume tended to be increased in the loaded groups. Mineral apposition and bone formation rates at the sutural surfaces were increased in the loaded groups ($P < 0.05$), but did not differ between loaded groups. These results indicate that relatively low loads (1 or 3 N) applied to rigidly integrated endosseous implants across an unfused suture are satisfactory for achieving expansion under the conditions of this study. The 3 N load resulted in slightly more expansion, but did not affect the rate of bone formation at the suture.

Key Words

Rigid endosseous implants • Anchorage • Expansion

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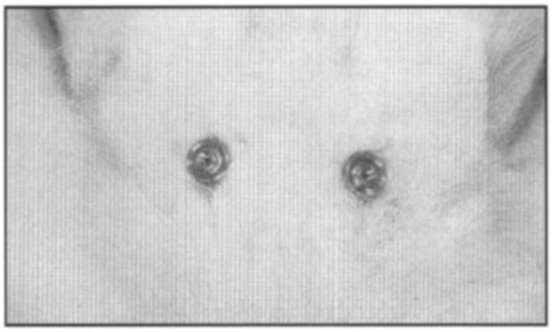

Figure 1

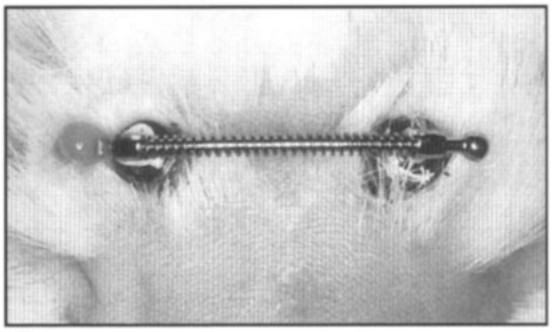

Figure 2

Figure 1
Location of implant placement in the rabbit's nose. Percutaneous abutments are in place on top of integrated implants.

Figure 2
Appliance used to provide expansion load to suture. An open coil spring has been compressed between the abutments to provide the expansion load. An .040 ball clasp has been used to hold the spring in place between abutments. Light cure resin ensures the ball clasp does not disengage the abutments.

The use of endosseous implants as abutments for sutural expansion would eliminate unwanted tooth movement and may allow nonsurgical treatment in cases with a compromised dentition. Rigidly integrated endosseous implants are ideal abutments for palatal expansion because they remain stable relative to supporting bone.[8-11] Indeed, endosseous implants provide rigid anchorage for orthodontic tooth movement experimentally[12-14] and clinically.[15-23] This study tests the potential of endosseous implants to resist orthopedic loading. Smalley et al.[1] and Turley et al.[24] reported the use of endosseous implants for facial orthopedic anchorage in monkeys. Turley et al.[24] placed bioglass-coated aluminum oxide implants in the anterior maxilla to provide suture expansion in monkeys. However, the results were compromised because the anchorage implants remained rigid in only one of the three monkeys. Smalley et al.[1] used titanium implants placed extraorally in the cranium, zygomatic bone, and maxilla of monkeys to support orthopedic protraction of the maxilla. Significant maxillary protraction was achieved in all four monkeys, and no mobility of the facial implants was seen. Movassaghi et al.[25] expanded the frontonasal suture of rabbits using titanium miniplates and screws to support the loading apparatus. Using cephalometry and direct measurements, they demonstrated significant expansion of this suture. Interestingly, the overall length of the skull was not changed.

Several investigators have examined sutural expansion with routine histology,[1,4,24,26,27] tritiated thymidine labeling,[6,28-30] and electron microscopy.[27] Although Guyman et al.[4] used fluorochrome labels to qualitatively describe bone formation in expanded sutures, no one has used intravital labels to quantitatively measure the dynamic histomorphometry of bone formation during sutural expansion.

The objectives of the present study were to evaluate: (1) the ability of rigidly integrated endosseous implants in the thin cortices of facial bones to support sutural expansion; (2) the dynamic histomorphometric parameters of the suture and adjacent bone; and (3) the osseous integration of the anchorage implants. We tested the hypothesis that rigidly integrated implants provide suitable anchorage for orthopedic expansion of facial sutures.

Materials and methods

Eighteen New Zealand White rabbits were used in this study. The rabbits were divided into three groups of six animals each: an unloaded control group, and two experimental groups. One experimental group was loaded with 1 Newton (N) and the other was loaded with 3 N of separating force. Each rabbit had two 4-mm flanged, commercially pure (cp) titanium implants (Nobelpharma USA, Inc, Chicago, Ill) placed bilaterally on the anterior surface of the snout on either side of the midnasal suture (Figure 1). All hair was removed from the nose, and vertical incisions were made over each implantation site. The periosteum was excised and the outer cortex perforated with a slow speed drill under copious saline irrigation. The inner cortex was then down-fractured with a titanium rod and the self-tapping implants screwed into place. Cover screws were placed and the skin was sutured over the implants. After 4 weeks of healing the implants were exposed and transcutaneous abutments placed. At the end of an additional 4-week healing period (8 weeks total), expansion appliances were placed on the abutments (Figure 2). The expansion appliance was constructed as follows: Two short pieces of .040" wire had round arch stops soldered to one end. These wires were cut to ~5 mm in length and cemented into the abutment with a glass ionomer cement. An .040" ball clasp was bent into an arc to fit through the arch stops with minimal friction. An open coil spring cut at an appropriate length to provide the desired expansion force (1 N or 3 N) was placed over the ball clasp, between the two tubes. The ball clasp was held in place by a small amount of light-cured acrylic on the end of the wire. The appliance placed on the control animals was identical to that placed on the experimental rabbits except the length of spring was adjusted so that no load was delivered between the implants. At the time of load placement, three implants in three separate rabbits were clinically mobile: two in the control group and one in the 1N group. Since an expansion load could not be placed, the rabbit in the 1N group was moved to the control group. Thus, the control group had 7 rabbits with 11 implants, the 1N group had 5 rabbits with 10 implants and the 3N group had 6 rabbits with 12 implants.

To allow the abutments to adapt and to minimize animal discomfort, loads above 1 N were applied in a stepwise fashion up to 3 N. Gradual loading of endosseous implants has been suggested as a way to improve the success of endosseous implants during the initial loading period.[31] In a previous study by Storey,[26] immediate loading of the premaxillary suture at ~2.5 N in rabbits caused significant discomfort, requiring the early euthanasia of one rabbit. Initially, both experimental groups received appliances with springs calibrated to deliver a load of 1 N (~ 102 g) to the implants. The springs were adjusted after 3 weeks to maintain a 1 N load for the 1N group or increased to 2 N (~204 g) for the 3N group. After an additional 3 weeks, the spring length was again adjusted in the experimental groups to maintain 1 N for the 1N group or increased to 3 N (~306 g) for the 3N group. Rabbits were given xylazine (2.5 mg/kg, IM, TID) after each load adjustment to minimize discomfort. The springs remained in place for the final 6 weeks of loading. The distance between the implants was measured twice daily for 4 days after load adjustment and weekly thereafter. As specified, multiple fluorochrome labels were injected to mark sites of bone mineralization (Table 1). After 12 weeks of loading, the rabbits were euthanized with an overdose of ketamine/xylazine and block specimens of the nasal bone with the implants and midline suture were recovered. Specimens were fixed in 70% ethanol and embedded in polyester resin. Sections (~ 100 μm) were cut through the center of both implants in the frontal plane with a diamond saw (Leitz 1600, Leica, Deerfield, Ill). Microradiographs were prepared of the most ideal midfrontal section and photomicrographs were obtained of the fluorescent and microradiographic images.

Stereologic point-hit and line-intercept methods were used to analyze the microradiographic and multiple fluorochrome histology of the suture.[32] Measurements were made within a microscopic field bordered by the outer and inner cortical surfaces and extending 2 mm on either side of the midnasal suture (Figures 3 and 4). Bone and sutural volumes were measured as percentages of the total tissue area from the microradiographic images. The mineral apposition rate (MAR) and bone formation rate (BFR) were measured at the sutural edge during the final 6-week loading period, corresponding to the maximum load in the 3N group. MAR was calculated using the line intercept method[32] by taking the average distance between the xylenol orange and alizarin complexone labels and dividing by the time between the labels. BFR was calculated using a point-hit method by measuring the volume of bone formed between the same two labels used for calculating MAR, and again dividing by the time between the two labels. The standard method for calculating BFR involves measuring the length of double- and single-labeled surfaces and then multiplying the sum of the double labeled surface plus one-half the single labeled surface times the MAR.[32] A point-hit method was used to measure BFR in this study for two reasons. First, the sutures are very tortuous, making it difficult to measure the length. Second, the entire length of the suture was double labeled in the loaded groups, a method which is amenable to a simple volumetric measurement.

Table 1
Schedule for loading and labeling

Time	Procedure / treatment
T minus 5 days	Tetracycline (10 mg/kg) IM bid x 3
T_0	Place implants
T_8 weeks	Place abutments
T_{12} weeks	Place 1 N loads in 1N and 3N groups Calcein green (5 mg/kg) IM bid
T_{15} weeks	Increase 3N group to 2 N Adjust 1N group to 1 N
T_{18} weeks	Increase 3N group to 3 N Adjust 1N group to 1 N Xylenol orange (60 mg/kg) IM bid
T_{22} weeks	Demeclocycline (10 mg/kg) IM bid
T_{23} weeks	Alizarin complexone (20 mg/kg) IM bid
T_{24} weeks	Tetracycline (10 mg/kg) IM bid
T_{24} weeks + 2 days	Euthanasia

Statistical analysis was performed using a one-factor analysis of variance (ANOVA) and a Student-Newman-Keuls post-hoc test. Percent data was converted by arcsin transformation to satisfy distributional assumptions of the ANOVA.[33]

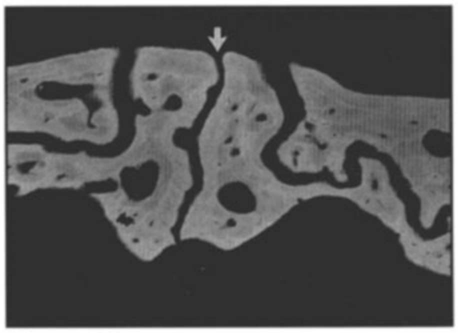
Figure 3A

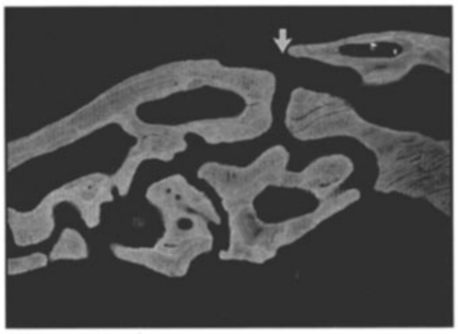
Figure 3B

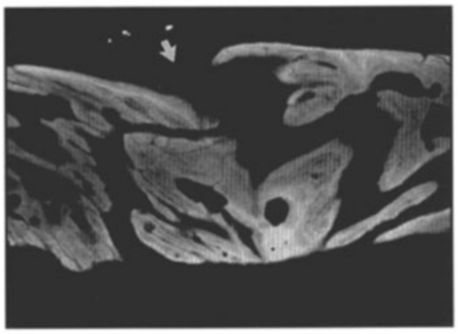
Figure 3C

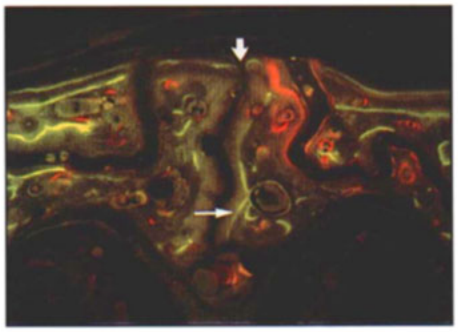
Figure 4A

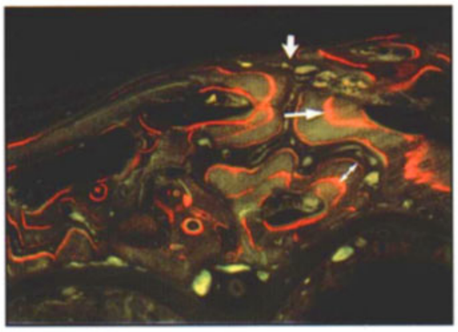
Figure 4B

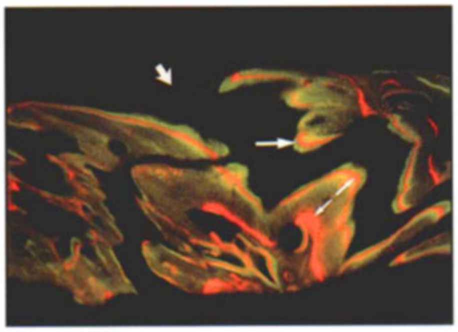
Figure 4C

Figure 3A-C
Microradiographic images of nasal suture from three groups. A: Unloaded control; B: 1N group; C: 3N group. Implants were located lateral to the suture (arrows). Sutures of the loaded groups are more tortuous than controls. Original magnification x37.

Figure 4A-C
Corresponding multiple fluorochrome images. A: Unloaded control; B: 1N group; C: 3N group. Bold arrows mark suture location. Horizontal arrows mark sharp label (A) due to lamellar bone formation and diffuse labels (B-C) due to woven bone formation. Small arrows mark two labels used to calculate MAR and BFR in loaded groups. Note relative absence of these labels at the sutural surface of the control. Original magnification x37.

Results

Three implant failures occurred prior to loading (during the healing period), resulting in an overall success rate of 92%. All 15 remaining implants remained stable during the loading period. The distance between the implants significantly increased ($p < 0.001$) in the loaded groups compared with the control, and was significantly higher ($p < 0.05$) in the 3N group compared with the 1N group (Figure 5).

Histologically, large areas of woven bone were formed at the sutural margins of the loaded groups (Figures 3 and 4). Broad diffuse fluorochrome labels marked woven bone mineralization at the time of injection (Figure 4). Conversely, the control group had no woven bone at the sutural margins, i.e., all labels were sharp (relatively narrow) indicating a much slower rate of mineralization (Figure 4). Percent bone measurements showed significantly less bone ($p < 0.05$) in the loaded groups compared with the control (Figure 6). Percent sutural volume increased in the loaded groups compared with the control, but was only significantly greater ($p < 0.05$) for the 3N group.

Both mineral apposition rate (MAR) and bone formation rate (BFR) were significantly increased ($p < 0.05$) in the loaded groups compared with the control (Figures 7). There was a trend toward an elevated MAR in the 3N group compared with the 1N group. However, there was no significant difference in MAR or BFR between the 3N and 1N groups.

Discussion

The 92% success rate for the present implants, placed in the thin cortical bones of the rabbit face, compares favorably with the success rates for implants placed in the mandibles of dogs[8] and humans.[11] All implant mobility occurred during the unloaded healing period, i.e., the implants failed to integrate. Once an implant was rigidly fixed within supporting bone, loading at a level sufficient to produce sutural expansion did not affect integration. This is an important result, because substantial sutural expansion was achieved: 6.8 ± 0.4 mm (mean ± SE) in the 3N group and 5.2 ± 0.6 mm in the 1N group. Daily and weekly measurements of suture width show a pattern similar to that seen in clinical suture expansion in which most of the expansion occurs within the first few days of loading, followed by a slow increase in the distance (Figure 8). The

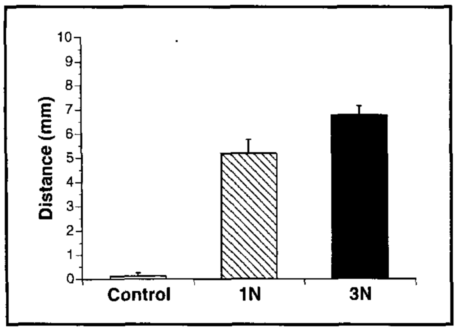

Figure 5

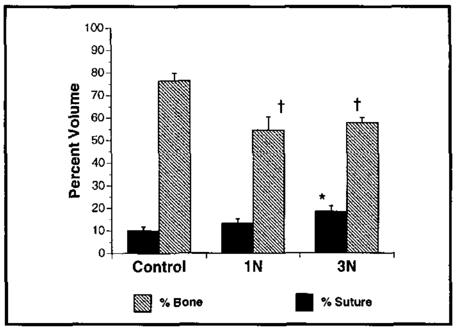

Figure 6

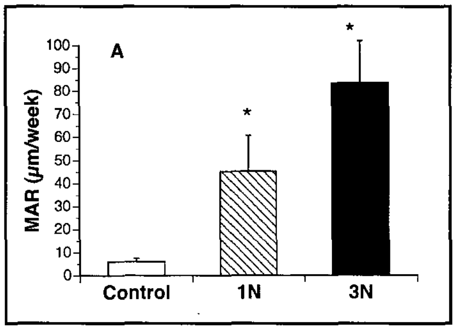

Figure 7A

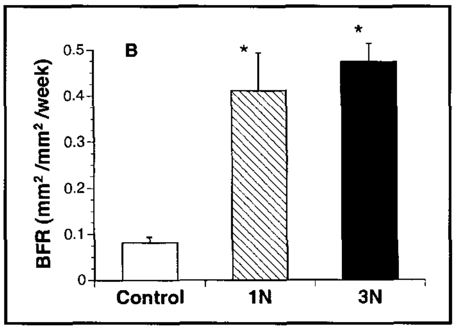

Figure 7B

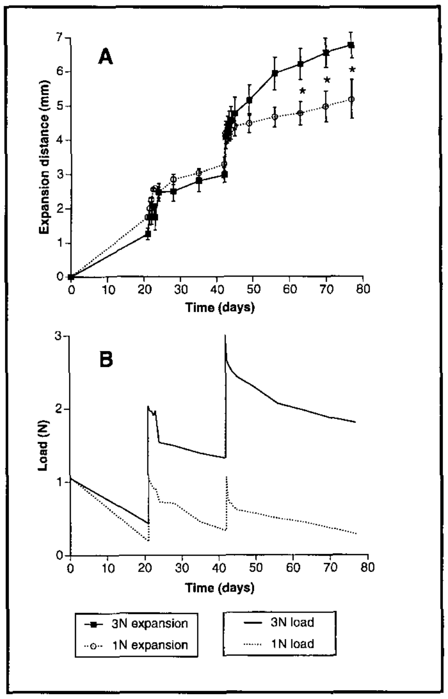

Figure 8

Figure 5
Expansion of suture expressed as the mean difference of initial and final measurements between implants for the three loading groups (Mean ± SEM, all groups significant at $p<0.05$).

Figure 6
Volume percent of suture and bone for three loading groups. (Mean ±SEM, * indicates significant difference in % suture from control at $p<0.05$, † indicates significant difference in % bone from control at $p<0.05$).

Figure 7A-B
A: Mineral apposition rate (MAR).
B: Bone formation rate (BFR) calculated at the suture during final 6 weeks of loading for three loading groups.
(Mean ± SEM, * indicates significant difference from control at $p<0.05$).

Figure 8A-B
A: Sutural expansion measured as increase in distance between implants. The slope of this curve is the rate of sutural expansion. * 3N is significantly greater than 1N at these time points ($p<0.05$).
B: Load on suture as function of time. Load was calculated using F=kx, where k is the spring constant and x is the distance between implants. As sutural expansion occurs, force decays. Loads were placed at day 0 and adjusted at days 21 and 42.

loaded groups showed similar patterns of expansion until the final 3 weeks, when significantly ($p < 0.05$) more expansion occurred in the 3N group. This study supports the hypothesis that endosseous implants can be used as anchorage abutments for sutural expansion.

The percent bone volume was decreased in the loaded groups compared with the controls. This result appears to conflict with the increase in MAR and BFR of the experimental groups. However, two additional observations help explain the apparent conflict. The first is the tendency for increased sutural volume of the loaded groups. Loading the suture expanded the soft tissue space, thereby decreasing bone volume (on a percentage basis). However, this increase in sutural volume was modest, and only statistically significant ($p < 0.05$) in the 3N group. A more important factor decreasing bone volume is that the new bone being formed at the sutural margins was of the woven type. Since there is more vascular (marrow) space within woven bone, the percent volume of new bone tends to be less for new bone compared with the original lamellar bone.

Both MAR and BFR were increased significantly in the loaded groups compared with the control. This increase is due directly to the activation of bone formation at the sutural surfaces. There was a trend for the 3N group to have an elevated MAR compared with the 1N group. The 3N group also showed a significantly ($p < 0.05$) greater amount of sutural expansion compared with the 1N group as measured by the increase in distance between the implants (Figure 5).

How the magnitude of the load affects the rate of sutural expansion and bone formation was an important objective of this research. During the initial phase of expansion, the rate of sutural separation relates to the stretching and tearing of the soft tissue of the suture.[27,34] Southard et al.,[28] using tensile loads across the interpremaxillary suture in rats, reported that the initiation of sutural expansion was rapid, occurring within several hours. Ten Cate et al.[27] reported that bone formation begins 3 to 4 days after applying sutural expansion loads in rat calvaria. Once the soft tissue of the suture has been maximally stretched, it is probable that further slow expansion is due to the bone formation rate at the suture margin that reestablishes the soft tissue connections within this bone.

Because there were two loaded groups, this study has a limited ability to characterize the effect of load level on sutural expansion rates. It was hypothesized that a higher load would result in increased MAR and BFR, reflecting more rapid sutural expansion. This view is supported by the larger sutural expansion in the 3N group compared with the 1N group (Figure 5) and the trend for an increasing MAR in the 3N group during the final loading period (Figure 7). However, when one compares the magnitude of the loads with the amount of sutural expansion and the MAR, a linear relationship seems unlikely. The increase in sutural expansion and MAR in the 3N group compared with the 1N group is small compared with the magnitude of load. Further support for a nonlinear relationship between load and sutural expansion is the fact that no difference in sutural expansion was seen between the two experimental groups during the intermediate 2 N load step in the 3N group (Figure 8). From a clinical perspective, these results suggest that lower expansion loads are indicated when implants are used as abutments.

A potential explanation as to why the sutural expansion and MAR were not as elevated as might be expected in the 3N group compared with the 1N group may reflect the type of bone being formed at the suture. As previously mentioned, the bone formed at the suture was mostly of the woven type (Figure 3). Turner et al.[35] proposed that woven bone formation occurs above a certain threshold of mechanical stimulus, and furthermore that woven bone formation is maximal above this threshold (i.e., an all-or-none response). Since both the 1N and 3N groups produced mostly woven bone at the suture, the amount of expansion and the MAR would not be expected to differ if woven bone is indeed formed by an all-or-none response. Our data showed that there was no significant difference in the histomorphometric parameters between the 1N and 3N groups (Figures 6 and 7). It is important to note that the hypothesis as to whether a threshold level for woven bone formation occurs in this model has not been adequately tested. A study with multiple groups at multiple load levels is required to determine if a threshold exists. However, since substantial woven bone formation occurred in the 1N group, any threshold must be below 1 N.

Other authors have looked at the effect of load level on sutural expansion.[6,26,28,30] The load levels in these studies ranged from 0.05 N to 3 N. Both Mörndal[29,30] and Zahrowski et al.[6] showed a plateau in the cellular response to sutural expansion using ^{3}H-thymidine labeling. Southard et al.[28] reported no difference in cellular labeling when the suture was loaded above 0.5 N. These results support the hypothesis that the response of bone to loading is saturable. However, it is important to realize that the labeling index used in these studies is not specific for osteogenic precursors.[36]

It is also important to note that the load levels reported in the present study represent the load applied to implants, teeth, or bone. The actual mechanical stresses and strains at the suture are unknown. Critical tissue strains depend on many factors, including sutural geometry, load level, abutment or tooth length (which act like levers), and implant placement (which affects load direction). Thus, attempting to quantitatively compare the sutural response to applied load between studies is difficult. The various experimental models as well as the type, placement, and design of the loading apparatus may all affect the levels of stress and strain within the suture.

Conclusion

Rigidly integrated endosseous implants are satisfactory abutments for sutural expansion under the conditions of this study. Ninety-two percent of the implants achieved integration and none failed during the loading period when ~5.2 to 6.8 mm of sutural expansion was obtained with loads of 1 N and 3 N, respectively. Since the MAR and BFR did not differ between the two loaded groups, the small increase in expansion seen in the 3N group is likely due to soft tissue changes. This is supported by the larger increase in suture volume in the 3N group. If woven bone formation is an all-or-none response, increasing loads significantly above the threshold for woven bone formation would not substantially affect the rate of bone formation, but would increase the stress on the implant abutments. Thus, our working hypothesis for the optimal load for implant anchored sutural expansion is the lowest load above the woven bone threshold that effectively separates the suture. Further studies are needed to determine the exact level of this load. These results indicate that relatively low loads (1 or 3 N) applied to rigidly-integrated endosseous implants across an unfused suture are satisfactory for achieving expansion under the conditions of this study.

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