Strength and Histological Characteristics of Periosteal Fixation to Bone After Elevation

Anthony P. Sclafani, MD; Michael S. Fozo, MD; Thomas Romo III, MD; Steven A. McCormick, MD

Background: Loss of brow elevation in the early postoperative period has been well documented; however, there has been no study quantifying the minimum time necessary to achieve lasting brow elevation. Previous work in our laboratory has demonstrated that complete re-adherence of periosteum to bone can take 12 weeks to occur after periosteal elevation. The correlation between adherence and the development of strength has never been examined.

Objective: To correlate histological characteristics of raised forehead periosteum with the strength of the periosteum-bone union postoperatively.

Subjects: Eighteen New Zealand white rabbits.

Methods: Rabbit foreheads were elevated in a subperiosteal plane and the flap reapproximated with chromic sutures. Animals were killed at 14, 28, 45, 63, or 84 days postoperatively, and all tissue superficial to the periosteum removed. The tension required to avulse sections of periosteum was then measured. Skulls were then sectioned and prepared for histological analysis of remaining periosteum. Avulsion forces and histological findings were compared with those unoperated-on controls.

Results: The forces necessary to avulse periosteum in the 14- and 28-day groups were significantly lower than for control animals; values at 45, 63, and 84 days were not significantly different from control animals. Healing periosteum displayed varying degrees of thickness, cellularity, edema, and vascular congestion. These features peaked at 28 days postoperatively then gradually resolved to near-control values by 84 days. Significant periosteal-to-bone contact did not appear until 45 days postoperatively.

Conclusion: Our results promote the use of methods of brow fixation that support mobilized soft tissues for a minimum of 6 weeks, until the elevated periosteum has significantly readhered to the underlying bone.

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ROW POSITION after subperiosteal endoscopic forehead surgery can be affected postoperatively by residual activity of brow (elevator and depressor) musculature and gravity until there is adequate strength of the periosteal attachment to the calvarium. Because of ease of use and low cost, temporary external (transcutaneous) screw fixation of brow position remains an integral part of the typical endoscopic brow-lift.

Recent histological evidence suggests that significant periosteal reattachment begins at 6 weeks postoperatively, but that nearly 12 weeks after surgery are necessary for raised periosteum to resemble that of unoperated controls. In clinical practice, transcutaneous screws are generally removed within the first 2 postoperative weeks. Inconsistent or poor long-term results after endoscopic brow surgery have been well documented. We believe this is at least partially attributable to loss of brow fixation before periosteal adhesion occurs when elevation is performed subperiosteally.

To test this theory, we used an animal model to examine both the biophysical properties and histological findings of periosteal adherence to bone that occurred as a function of time after periosteal elevation. Strength of bone-periosteal adherence and histological findings were then compared with those of unoperated-on controls.

METHODS

Eighteen female ex-breeder New Zealand white rabbits (Hare-Marland, Hewitt, NJ) were chosen for their large cranial surface area. Three animals were included in each experimental group, as well as in the control group, and each was given subcutaneous flocillin prior to surgery.
Calvarial Periosteal Avulsion Forces

<table>
<thead>
<tr>
<th>Time After Periosteal Elevation, d</th>
<th>Mean (SD) Force Required to Avulse Periosteum, N</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>2.06 (0.65)</td>
<td>.004</td>
</tr>
<tr>
<td>28</td>
<td>2.12 (0.50)</td>
<td>.008</td>
</tr>
<tr>
<td>45</td>
<td>3.14 (1.42)</td>
<td>.95</td>
</tr>
<tr>
<td>63</td>
<td>2.75 (0.74)</td>
<td>.31</td>
</tr>
<tr>
<td>84</td>
<td>3.34 (0.72)</td>
<td>.70</td>
</tr>
<tr>
<td>Controls</td>
<td>3.17 (1.19)</td>
<td>. . .</td>
</tr>
</tbody>
</table>

*P value refers to comparison of group to control values using unpaired t test.

The research protocol was approved by the Institutional Animal Care and Use Committee, the Department of Comparative Medicine and the Office of Research Administration of New York Medical College, and animal care was in accordance with institutional policies for vertebrate animals.

**Surgery**

Each rabbit was anesthetized with intramuscular xylazine hydrochloride and ketamine hydrochloride. The forehead was shaved, prepared, and draped in standard sterile fashion. One percent lidocaine with 1:100,000 epinephrine was infiltrated subcutaneously along the future incision. A vertical, lateral incision was made down to bone. The periosteum and all superficial soft tissues were raised as one unit completely across to the opposite side of the forehead. A butterfly drain was then placed beneath the periosteum, which was then closed with interrupted 4-0 chromic sutures. Skin was closed with interrupted 4-0 chromic sutures. Antibiotic ointment was placed along the incision, and the animals were kept in Elizabethan collars for 5 days. Drains were attached to red-top vacuum test tubes for 24 hours, then removed. Depending on the experimental group, animals were killed with sodium pentobarbital collars for 5 days. Drains were attached to red-top vacuum test tubes for 24 hours, then removed. Depending on the experimental group, animals were killed with sodium pentobarbital.

**Biophysical Properties**

A template was used to mark out the forehead into 2 areas: the right side was used for a series of tension measurements while the left was kept intact for histological analysis. Depending on the available area, three to five 1 X 1-cm periosteal squares were outlined on the right forehead and then sharply incised with a No. 11 scalpel. Care was taken to sever the perforating arteries medial to each orbit. Hemostasis was then carefully attached to a corner of the first periosteal square. A tensiometer (Mark 10 Digital Force Gauge; Chatillon Scales and Force Measuring Instruments, Kew Gardens, NY), set to record the maximum tension, was then attached to the hemostat with silk suture. With the rabbit head fixed, the tensiometer was gently raised perpendicular to the forehead. Force was gradually increased and maintained until the periosteum began to peel away from the underlying bone. After a periosteal square was completely avulsed, the maximum force generated was recorded and the process was repeated again for the next square.

**Histological Assessment**

A sagittal saw was used to section the portion of left forehead with undisrupted periosteum. This specimen was immediately placed in a labeled container of 10% formalin for 1 week, followed by 3 weeks of decalcification in 5% nitric acid. Each specimen was then embedded in paraffin and sectioned before staining with hematoxylin-eosin.

Slide preparations of tissue samples were examined at X100, X200, and X400 magnification by 2 independent blinded observers. Periosteal characteristics such as vascular congestion, edema, cellularity, and thickness were noted. Degree of bone-periosteum interface and the degree of inflammatory cell infiltrate were estimated.

**Statistical Analysis**

The mean, median, range, and SD of tension values for each group of animals were calculated. Data were analyzed by an unpaired t test to determine statistical significance of tension measurements.

**Results**

**Tension Measurements**

The mean (SD) of tension measurements for each time period are displayed in the Table. The mean force required to avulse unoperated periosteum was 3.17 N (SD, 1.19 N). In the operated-on animals, mean tension gradually increased from 2.06 N (SD, 0.65) at 14 days postoperatively to 3.34 N (SD, 0.72 N) by 84 days. Only the tension values 14 and 28 days postoperatively were significantly less (P < .009) from controls, however. There was a statistically significant increase in mean force required between 28 days (2.12 [0.50] N) and 45 days (3.14 [1.42] N) (P = .03) (Figure 1).

**Histological Findings**

No significant differences in histological appearance were appreciated among the 3 animals in a given time group (Figure 2).

Control animals exhibited complete, uniform, and smooth periosteal adhesion to bone. There were no inflammatory cells. The periosteum was thin, lacking vascular congestion or edematous changes. By 14 days after periosteal elevation, periosteum was more than 90% dissociated from underlying bone. Isolated small islands of bone remodeling were seen extending toward the periosteum. Both periosteal cellularity (fibroblasts) and thickness were nearly double that of control animals. Periosteal capillaries were markedly increased in both number and prominence. At 28 days after surgery,
periosteal cellularity remained constant; however, the overall periosteal thickness increased as did the number of capillaries. The frequency of inflammatory cells also increased from the 14-day group. For the first time, scattered osteoblasts appeared along the surface of bone. Histological periosteal adherence remained rare, at approximately 20% contact. At 45 days, significant periosteal adherence (approximately 50%-60%) was seen, as was a generalized (rather than scattered) distribution of osteoblasts. Periosteal cellularity, thickness, and vascular congestion all decreased from the 28-day group but remained far greater than control values. At 63 days, periosteal-bone molding and uniform adherence were seen for the first time. The periosteum remained thick and hypercellular with an irregular bony interface, distinguishing this time group from controls. Finally, at 84 days, periosteal cellularity was significantly less that at 63 days. Much less activity was seen in this group. Inflammatory cells were no longer present and the periosteum was more compact. The interface was smooth with near-complete adherence. Edema and vascular congestion likewise resolved. Apart from a slightly increased thickness and minor foci of nonunion, these animals were nearly indistinguishable from controls.

Subperiosteal elevation in endoscopic brow-lifting is frequently cited as providing an enhanced optical cavity as well as a relatively inelastic platform (periosteum) for brow and forehead mobilization. Fixation can be performed with a number of different techniques. Temporary transcutaneous screws are an inexpensive and common method of brow fixation in the early postoperative period. Removal of transcutaneous brow fixation screws in the first 2 weeks after surgery has been advocated by surgeons who cite generally good results, but loss of brow elevation in the early postoperative period has been described. Some authors have recommended routine overcorrection, an implicit admission that some postoperative “settling” of brow position does occur. There has been only one study to date that has examined periosteal adhesiveness after forehead lifting. This study used 60 adult white mice and measured flap adhesiveness 2 to 10 days after a subperiosteal or subgaleal coronal forehead lift. Control animals were not used, nor were time periods longer than 10 days. While the relative strengths of periosteal adhesion for these time periods were described, few conclusions could be drawn without comparison to control animals. Romo et al examined the histological appearance of periosteal refixation after endoscopic or bicoronal forehead lifts. Significant periosteal adherence was not seen until 6 weeks, with 12 weeks being required before complete union to bone was noted. However, histological apposition could not be assumed to correlate with periosteum-bone bond strength. The present study addressed those concerns by evaluating strength and histological characteristics side by side.
We chose female ex-breeder rabbits for their large forehead area. A template was created that standardized all cuts in order to reduce interanimal variability. The same corresponding areas of periosteum were tested in each animal. Likewise, the same portion of skull was sectioned for histological assessment in each animal.

The use of many periosteal squares for each animal allowed us to look at how specific portions of the brow healed relative to each other. Tension values for the upper brow tended to be slightly higher than those in inferior locations, but these differences were not statistically significant.

There was minimal interanimal variability in histological appearance for each time period. This is probably a reflection of the wide time interval spacing. As time increased postoperatively, groups displayed an orderly progression of periosteal thickness, cellularity, vascular congestion, inflammation, and edema. These features peaked at 28 days, then slowly returned to values similar to controls by 84 days.

There was minimal periosteal-to-bone contact in all 6 animals of the 14- and 28-day groups, whereas significant but variable adhesion was seen in all 3 animals at 45 days. Every animal in the 63- and 84-day groups displayed near uniform union of periosteum to bone. These findings correlated well with those of the previous study. This degree of periosteum-to-bone contact correlated well with tension measurements. While mean tension measurements displayed a rising trend from 14 days to 84 days, only the 14- and 28-day groups evidenced statistically significantly lower values compared with controls. These were the only 2 groups with minimal periosteal-to-bone interface. Interestingly, although periosteum was only about 50% adherent to bone at 45 days, tension values for these animals were not significantly lower than for the 63- and 84-day groups, which evidenced near-complete union (3.14 vs 2.75 and 3.34 N, respectively). Other characteristics of the healing process did not correlate with adhesion. Inflammatory cell infiltrate, periosteal edema, and vascular congestion all peaked at 28 days despite the low tension measured in this group.

While the amount of time necessary to regain control values of periosteal strength is suggested, we do not know what fraction of strength is actually required to maintain brow position postoperatively. Despite this, the minimal contact between periosteum and bone and weak periosteal attachment seen at 4 weeks postoperatively raises significant doubt as to the efficacy and efficiency of using fixation techniques that support the brow for only 7 to 14 days in providing lasting brow elevation. The use of temporary transcutaneous screws for 1 or 2 weeks postoperatively should probably be reevaluated. By contrast, any fixation method that offers support for at least 6 weeks (eg, cortical tunnels, permanent titanium tacks, or permanent sutures tied to small titanium plates secured to the calvaria) would appear to provide soft tissue support for an adequate period of time. While the periosteum-bone interface was not completely developed at 45 days, this attachment was not significantly weaker than that of control animals.

Many methods for brow fixation after endoscopic brow-lift have been described, but little is known about the processes that occur during long-term healing of elevated periosteum. This is the first study to compare the long-term biophysical properties of previously raised brow periosteum with histological characteristics of that periosteal-bone interface. Periosteal strength correlated well with the degree of bony adhesion seen under the microscope. Almost no adhesion is seen at 14 or 28 days postoperatively, which correlates well with the significantly lower tensions required to avulse sections of periosteum in these animals. Approximately 45 days are necessary for periosteal strength to reach control values in a rabbit model. Histologically, this strength is associated with a resolving inflammatory phase and active osseogenic phase. To ensure maintenance of brow position following surgery, fixation should last at least until those processes have occurred: 6 weeks in an animal model and presumably a similar duration in humans.

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Corresponding author and reprints: Anthony P. Sclafani, MD, Division of Facial Plastic Surgery, The New York Eye & Ear Infirmary, 310 E 14th St, Sixth Floor, North Building, New York, NY 10003 (e-mail: asclafani@nyee.edu).

REFERENCES