Ethylene oxide gas sterilization: a simple technique for storing explanted skull bone

Technical note

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The authors evaluated the effectiveness of a simple technique using ethylene oxide (EtO) gas sterilization and room temperature storage of autologous bone grafts for reconstructive cranioplasty following decompressive craniectomy. The authors retrospectively analyzed data in 103 consecutive patients who underwent cranioplasty following decompressive craniectomy for any cause at the University of Illinois at Chicago between 1999 and 2005. Patients with a pre-existing intracranial infection prior to craniectomy or lost to follow-up before reconstruction were excluded. Autologous bone grafts were cleansed of soft tissue, hermetically sealed in sterilization pouches for EtO gas sterilization, and stored at room temperature until reconstructive cranioplasty was performed.

Cranioplasties were performed an average of 4 months after decompressive craniectomy, and the follow-up after reconstruction averaged 14 months. Excellent aesthetic and functional results after single-stage reconstruction were achieved in 95 patients (92.2%) as confirmed on computed tomography. An infection of the bone flap occurred in eight patients (7.8%), and the skull defects were eventually reconstructed using polymethylmethacrylate with satisfactory results. The mean preservation interval was 3.8 months in patients with uninfected flaps and 6.4 months in those with infected flaps (p = 0.02). A preservation time beyond 10 months was associated with a significantly increased risk of flap infection postcranioplasty (odds ratio [OR] 10.8, p = 0.02). Additionally, patients who had undergone multiple craniotomies demonstrated a trend toward increased infection rates (OR 3.0, p = 0.13).

Data in this analysis support the effectiveness of this method, which can be performed at any institution that provides EtO gas sterilization services. The findings also suggest that bone flaps preserved beyond 10 months using this technique should be discarded or resterilized prior to reconstruction. (DOI: 10.3171/JNS-07/08/0440)

Key Words • decompressive craniectomy • ethylene oxide • skull bone flap • sterilization

In patients receiving extensive decompressive craniotomies, it is generally preferred to use the removed autologous bone flap for later reconstruction because of its mechanical, immunological, and aesthetic advantages. Conventional methods of preserving the skull grafts have involved subcutaneous implantation in the abdomen, thigh, or scalp. However, this method of storage can cause patient discomfort and necessitates the creation of a second incision site, thereby prolonging surgery and introducing the risk of postoperative complications such as wound infection at this second site. Alternatively, the bone flap can be cryopreserved alone, sterilized in a steam autoclave prior to cryopreservation, or immersed in hydrogen peroxide before EtO gas sterilization with storage at operating room temperature. Ethylene oxide sterilizes through its properties as a strong alkylating agent, reacting with proteins and nucleic acids in a temperature-dependent fashion to kill microorganisms. We describe a simple and rapid technique for EtO gas sterilization of bone flaps, which has been used at our institution since 1996 and produces excellent aesthetic and functional results in skull reconstruction and low overall infection rates.

Materials and Methods

Data Collection

We retrospectively reviewed the medical records of 216 consecutive patients who underwent decompressive craniectomy for various indications between March 1999 (conversion to computerized medical records) and July 2005, as approved by the institutional review board protocol #2005-0036. Of these patients, 103 eventually underwent cranioplasty with autologous bone flap between May 1999 and October 2005 (Table 1). Five patients moved out of state during the follow-up period, and bone flaps were given to...
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### Table 1

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of Patients</th>
</tr>
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<tbody>
<tr>
<td>aneurysmal SAH</td>
<td>74</td>
</tr>
<tr>
<td>head trauma w/ epidural, subdural, or intracranial hemorrhage</td>
<td>8</td>
</tr>
<tr>
<td>tumors w/ hemorrhage or intractable cerebral edema</td>
<td>8</td>
</tr>
<tr>
<td>cerebral infarction, thromboembolic or hemorrhagic ruptured AVM</td>
<td>6</td>
</tr>
<tr>
<td>ruptured AVF</td>
<td>2</td>
</tr>
</tbody>
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* AVF = arteriovenous fistula; AVM = arteriovenous malformation.

Results

One hundred three consecutive patients underwent reconstructive cranioplasty an average of 4 months after craniectomy (range 9 days–15 months). Aesthetic and functional results of the cranioplasty were assessed based on the bone contour on CT scans and cosmetic appearance on physical examination. During a postoperative follow-up period averaging 14 months after cranioplasty (range 1–63 months), 95 of the patients (92.2%) had excellent aesthetic and functional results after a single-stage unsupplemented reconstruction, as judged by CT studies and physical examination (Figs. 2 and 3). After a decompressive craniectomy, a wound infection unrelated to the removed bone flap developed in one patient, who was later able to undergo successful reconstruction with autologous bone after eventual resolution of the infection. Another patient required drainage of a subdural fluid collection under the replaced flap, related to shunt malfunction. In eight patients (7.8%) re-planted bone flaps became infected, and pus was observed at the time of flap removal; most samples sent from the operating room grew staphylococcus (Table 2). These skull defects were eventually reconstructed using PMMA with satisfactory results.

The present study mostly consisted of cases (72%) involving aneurysmal SAH and included only a few patients with known risk factors for impaired postoperative wound infection and poor healing such as diabetes, immunosuppression, malnutrition, and malignancy. Multiple craniotomies and an increased bone flap preservation interval between craniectomy and reconstruction appeared to be associated with an increased risk for flap infection (Table 3). There was no significant difference in age distributions between the infected and uninfected patients, but the group with infected bone flaps after cranioplasty had longer mean flap preservation intervals (p = 0.02). When the infected
and uninfected groups were divided into those with flap preservation intervals less than 10 months and those with intervals of 10 or more months, the latter group had a significantly higher risk of infection (OR 10.8, p = 0.02). Patients who underwent multiple craniotomies demonstrated a trend toward increased infection rates with an OR of 3.0, but this association did not reach statistical significance (p = 0.13). Notably, infections occurred in two patients with neuropsychiatric issues who had chronically scratched their incision sites despite all reasonable preventative efforts by their families.

**Discussion**

We describe a technique of EtO gas sterilization that is simple and effective. As mentioned, successful one-stage reconstruction was achieved in 95 patients (92.2%) without supplementation by alloplastic material in a series of 103 consecutive patients. During the average 14-month follow-up, the 95 uninfected patients showed good bone contour on CT without significant bone resorption. Eight patients (7.8%) had bone flap infections postcranioplasty requiring flap removal and eventual PMMA reconstruction with satisfactory results. Among these eight patients were two with the unusual added risk factor of chronic incision scratching due to neuropsychiatric issues.

To the best of our knowledge, EtO gas sterilization has been reported in only a small series of 16 patients who had good aesthetic and functional outcomes; flap infection occurred in one patient (6.2%). However, Missori et al. used a technique that included 30% hydrogen peroxide immersion for 24 hours prior to EtO gas sterilization. Given that the results of using gas sterilization alone were comparable, hydrogen peroxide immersion does not appear to be an essential component.

Because subcutaneous pocketing and cryopreservation are the most commonly used methods for autologous bone flap preservation, we compared results from our present analysis with those from studies focused on these alternative techniques. Data from a metaanalysis of cryopreservation of autologous bone flaps at −16 to −40°C compared with abdominal subcutaneous pocketing showed no significant difference between the infection and resorption rates among the seven analyzed studies. However, the authors asserted that flap freezing obviated the need for an additional surgical site and increased surgical time, thereby intuitively reducing infection rates, which would also hold true for EtO gas sterilization or other techniques that do not involve subcutaneous pockets.

Movassaghi and associates conducted a retrospective analysis of 53 consecutive patients who had undergone cranioplasty after emergency decompressive craniectomy with

![Fig. 2. Axial CT scans obtained in a 42-year-old man with SAH due to a ruptured anterior communicating artery aneurysm, who underwent left peritonal craniectomy with reconstructive cranioplasty at 5 months after initial decompressive craniectomy. A: Preoperative scan showing a Fisher Grade 3 SAH. B: Postoperative scan demonstrating brain tissue bulging through the craniectomy opening from increased intracranial pressure. C: Follow-up scan revealing reduction of cerebral edema and resolution of sulcal effacement. D: Scan obtained after cranioplasty, showing replacement of the bone flap isodense with adjacent skull bone.](image1)

![Fig. 3. Axial CT scans obtained in four patients after reconstructive cranioplasty for a bone defect following right-sided peritonal decompressive craniectomy, revealing bone flaps isodense with adjacent skull bone and a satisfactory contour.](image2)
the bone flap stored in an abdominal subcutaneous pocket. Successful one-stage reconstruction was achieved in 49 patients (92.5%) and supplementation with alloplastic material was required in eight patients (15.1%) to accomplish the desired contour. There were three infections (6.1%), one graft being infected in the abdominal pocket on retrieval and two infections occurring after cranioplasty. Tybor and colleagues described a series of 36 patients undergoing cranioplasties; of course, different series may consist of varying proportions of higher-risk patients such as those with diabetes, immunosuppression, malignancy, trauma, multiple same-site operations, and prolonged surgeries. In addition, (25.9%) of 54 patients. Nagayama and colleagues described a technique for preserving autologous bone flaps through immersion in 200 mg of amikacin and cryopreservation at −16°C in 206 patients between 1980 and 1998. Their decompressive craniectomy study consisted mostly of patients with aneurysmal SAH (48%), followed by intracranial hemorrhage (23%), head trauma (14%), and cerebral infarction (12%). They noted bone flap infections in eight patients (3.9%) and found that the bone preservation period averaging 31.1 days in the infected group was shorter than the average 54.9 days in the uninfected group (p < 0.05). In contrast, with our technique the flap preservation interval of 6.4 months in the infected group was significantly longer than the 3.8 months in the uninfected group (p = 0.02).

Admittedly, comparisons of graft infection rates can be challenging because the rates can vary widely among neurosurgical case series, even those involving clean cranioplasties; of course, different series may consist of varying proportions of higher-risk patients such as those with diabetes, immunosuppression, malignancy, trauma, multiple same-site operations, and prolonged surgeries. In addition,

### Table 2

<table>
<thead>
<tr>
<th>Age (yrs), Sex</th>
<th>Indication for Surgery</th>
<th>Cranectomy Procedure</th>
<th>Flap Preservation Interval (mos)</th>
<th>Risk Factors</th>
<th>Time to Removal of Infected Flap (mos)</th>
<th>Culture Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>41, M</td>
<td>blunt head trauma w/ SDH</td>
<td>rt FTP w/ partial fronto-temporal lobectomy</td>
<td>11</td>
<td>preservation interval ≥10 mos</td>
<td>3</td>
<td>no growth</td>
</tr>
<tr>
<td>60, M</td>
<td>ruptured dural AVF</td>
<td>lt FTP w/ evacuation of ICH</td>
<td>0.5</td>
<td>multiple craniotomies</td>
<td>6</td>
<td>coag-neg staph</td>
</tr>
<tr>
<td>52, F</td>
<td>lt ICA stroke after CEA</td>
<td>lt TPO w/ evacuation of ICH</td>
<td>6</td>
<td>chronic incision scratching</td>
<td>5</td>
<td>no growth</td>
</tr>
<tr>
<td>61, F</td>
<td>ruptured ACoA aneurysm</td>
<td>rt ptetorional bicoronal</td>
<td>1</td>
<td>none</td>
<td>3</td>
<td>coag-neg staph</td>
</tr>
<tr>
<td>35, M</td>
<td>recurrent craniopharyngi-oma</td>
<td>rt ICH</td>
<td>13</td>
<td>preservation interval ≥10 mos, multiple craniotomies</td>
<td>1</td>
<td>coag-neg staph</td>
</tr>
<tr>
<td>27, F</td>
<td>ruptured rt MCA aneurysm</td>
<td>rt FTP</td>
<td>14</td>
<td>chronic incision scratching, preservation interval ≥10 mos</td>
<td>1</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>48, F</td>
<td>ruptured lt MCA bifurca- tion aneurysm</td>
<td>lt ptetorional</td>
<td>5</td>
<td>multiple craniotomies</td>
<td>6</td>
<td>coag-neg staph</td>
</tr>
<tr>
<td>22, F</td>
<td>ruptured MCA aneurysm w/ ICH</td>
<td>lt ptetorional</td>
<td>1</td>
<td>multiple craniotomies</td>
<td>1</td>
<td>coag-neg staph</td>
</tr>
</tbody>
</table>

* ACoA = anterior communicating artery; CEA = carotid endarterectomy; coag-neg staph = coagulase-negative staphylococcus; FTP = frontal-temporal-parietal; ICA = internal carotid artery; ICH = intracerebral hematoma; MCA = middle cerebral artery; SDH = subdural hematoma; TPO = temporal-parietal-occipital.

### Table 3

**Comparison of infected and uninfected groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infected</th>
<th>Uninfected</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>total no. of cases</td>
<td>8</td>
<td>95</td>
<td>0.46</td>
</tr>
<tr>
<td>mean age (yrs)</td>
<td>43.3 ± 5.1</td>
<td>46.7 ± 1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>mean flap preservation interval (mos)</td>
<td>6.4 ± 2.0</td>
<td>3.8 ± 0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>interval ≥10 mos</td>
<td>3</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>multiple craniotomies</td>
<td>4</td>
<td>24</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Mean values are presented ± standard deviations.
postoperative wound infection rates vary slightly during different time periods within an institution and between institutions, and the surgeon’s diagnosis of wound infection can affect the reported wound infection rates at any center. Moreover, data in various studies have shown a range for postcraniotomy infections from 1.7 to 9.7%, depending on the type of antibiotic prophylaxis.

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Despite these challenges, we identified possible modifiable risk factors associated with flap infection in our series to improve our practice. As noted, two patients with infections had chronically scratched their incisions; additional precautions in protecting the incisions and instruction to families of future patients with similar neuropsychiatric problems will be essential. The most significant modifiable risk factor in the present series was the flap preservation interval. Bone flaps were preserved for up to 24 months following craniectomy, and cranioplasty was performed as late as 15 months after the original bone flap removal. Attempts to replace the bone flap were generally made within 3 months of discharge; at times, delays occurred because of missed follow-up appointments or other extraneous factors. Of the eight patients who underwent reconstruction at 10 to 15 months after craniectomy, only five were infection free at an average of 8 months’ follow-up (range 2–21 months), for an infection rate of 38% within this subset. We calculated an OR of 10.8 for a bone flap preservation interval of 10 or more months. Thus, infection rates can be diminished by a change in our current practice—to resterilize or discard autologous bone flaps requiring storage beyond the 10-month interval or to strictly enforce an early follow-up.

A limitation of ETO sterilization at this time is the reduced availability of this service at some US institutions due to the increasingly stringent work-exposure standards imposed by the OSHA. The ETO itself is inexpensive and can be used in small amounts to sterilize medical instruments and human tissues, especially those that cannot withstand steam autoclaving. Medical products or implants that contain plastic must be sterilized with ETO because they can be permanently damaged by steam or radiation. In addition to its applications in the medical industry, ETO sterilization is used in the food and contact lens industries, and there are thousands of industrial products that specifically require ETO sterilization. After the gas sterilization process, ETO is extensively flushed and aerated so that there is minimal threat of exposure for those operating the sterilizers and an increased cost of engineering devices to monitor or control exposure. In the 2005 regulatory review of OSHA’s ETO standard, a marked reduction in the number of hospitals using ETO was reported, probably because of the challenges in complying with the strict OSHA standards regarding exposure limits for hospital workers who operate the sterilizers. There have been engineering control technologies that help reduce work exposures, but these remain less than ideal. In the future, engineering technology may improve to the extent that work exposure can be inexpensive and effectively minimized, allowing this bone flap preservation technique to be used more widely.

Conclusions

Our analysis of outcome and infection rates supports the use of gas sterilization as a simple method of bone flap preservation following craniectomy and is available to any institution with ETO gas sterilization services.

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