Cell Tissue Banking (2007) 8:243–246
DOI 10.1007/s10561-006-9032-x

Deep-freeze preservation of cranial bones for future cranioplasty: nine years of experience in Soroka University Medical Center

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Received: 2 October 2006 / Accepted: 15 November 2006 / Published online: 2 February 2007

Abstract
Background Decompressive craniectomy is routinely performed in many neurosurgical centers to treat intracranial hypertension refractory to medical therapy as a result of head trauma, CVA or various brain tumors. When the patient survives his illness, cranioplasty with autologous bone graft or other reconstructive materials is considered to repair the skull defect.
Objective This prospective study reviews the cases of decompressive craniectomies followed by later cranioplasty undertaken at our institute through the years 1996 and 2005 and describes the method used for preservation of removed bone flaps for future cranioplasty.
Subjects and methods Sixty-eight patients underwent decompressive craniectomies since 1996. A protocol was designed to prepare the removed bone flaps for deep freeze preservation. After removal, the bone flaps were transferred to the skin bank at our institution within 6 h, gently rinsed using 1–3 liters of sterile saline (0.9% NaCl) supplemented with antibiotics (neomycin, 2 mM) with no dimethylsulfoxide (DMSO), then flaps were wrapped in two layers of sterile plastic coverage and preserved at –80°C.
Results The patient's population will be presented. Since 1996 we have performed 12 cranioplasties using deep-freeze preserved autologous bone graft. It took a rather long learning period, beginning with a single patient per year and continued with several others. Up to now, no case of infection, osteomyelitis or bone resorption following cranioplasty have occurred.
Conclusion Deep-freeze preservation of autologous bone grafts to reconstruct skull defects after decompressive craniectomy is a useful procedure and has a low revision rate.

Keywords Autologous bone grafts · Decompressive craniectomy · Deep-freeze preservation

Abbreviations

DMSO	Dimethylsulfoxide
OR	Operating room

N. Grossman: deceased 23 December 2006.

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Introduction

Decompressive craniectomy is routinely performed in many neurosurgical centers to treat intracranial hypertension refractory to medical therapy as a result of head trauma, CVA or various brain tumors. When the patient survives his illness, cranioplasty with autologous bone graft or other reconstructive materials is considered to repair the skull defect. Autologous bone flaps, which are still some of the most commonly used materials for delayed cranioplasties, were first reported in the 1950s; although the results were mostly satisfactory, flap resorption, osteomyelitis or infection were occasionally observed (Abbot 1953; Elliott and Scott, 1951; Odom et al. 1952). Nevertheless, autologous bone flaps obtained during the initial operation possess features that make them excellent for later cranioplasty; they are viable, exhibit perfect fit and present no risk of disease transmission. We reviewed the clinical and aesthetic results obtained for our patients who underwent decompressive craniectomy and delayed cranioplasties, to reevaluate the efficacy of autologous bone flaps and to elucidate important points for the successful application of this conventional method.

Subjects and methods

Sixty eight patients underwent decompressive craniectomies performed at our institute (Fig. 1).

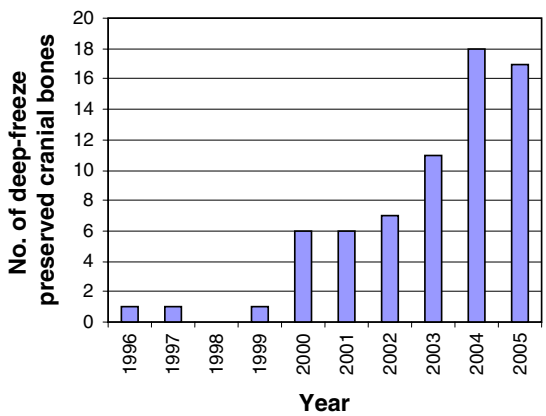

Fig. 1 Number of deep-freeze preserved bone flaps through the years 1996 and 2005

Since 1996, 12 patients underwent decompressive craniectomies with later autologous bone graft cranioplasties. The initial indication for decompressive craniectomy was head trauma in majority of the patients, intracerebral hemorrhage and CVA. After cranioplasty, physicians monitored complications during outpatient visits for at least 12 months. Patient ages ranged from 1 to 63 years (mean, 25.9 year); five patients were younger than 20 years. Table 1 summarizes the patients' age and sex, the duration of deep-freeze preservation, the post-operative follow-up period, and the complications.

Following removal, the bone flaps were transferred to the skin bank at our institute and processed within 6 hours. They were gently rinsed with 1–3 liters of saline supplemented with antibiotic (neomycine, 2 mM) with no dimetylsulfoxide (DMSO). The bones were wrapped using two layers of sterile plastic coverage and stored in a deep freezer at –80°C, for periods ranging from 0.25 to 27 months (mean, 9.25 months). On the morning of cranioplasty, the bags containing the bone flap were removed from the freezer and transferred to the O.R. stored in an icebox. The thawed bone flap was removed from the bags and washed in sterilized saline solution. After reopening of the wound, the bone flap was fixed in its original position, contacting the edge of the bone defect as closely as possible, with craniofix or titanium plates. Antibiotics were intravenously administered during the procedure.

Results

All grafts were successfully re-implanted with no complications observed during follow-up monitoring. There was no bone flap resorption, osteomyelitis or infection on post-operative follow up period, and the aesthetic results were satisfactory (see Table 1).

Discussion

Several methods for preserving bone flaps for delayed cranioplasty have been suggested (Abbot 1953; Ackigoz et al. 1986; Assano et al. 1953; Itoh 1991; Korfali and Askoy 1988; Kurokawa et al.

Table 1 Summary of patients who underwent autologous bone flap cranioplasty

	Freezing temperature –80°C
No. of patients	12
Age (year) (mean/range)	25.9/1–63
Male:Female	10:2
Duration of deep-freezing (months) (mean/range)	9.2/0.25–27
Post-operative follow up period (months)	12
Complications	None

1995; Nakajima et al. 1977; Odom et al. 1952; Osawa et al. 1990; Ozaki 1994; Pasaoglu et al. 1996; Prolo et al. 1979; Vanclocha et al. 1997; Yamashita et al. 1992). These methods may be divided into two categories, i.e., those that preserve the bone flap in the patient's body and those that store the flap extracorporeally. Nakajima et al. (1977) reported a method of subcutaneous preservation in the thigh, and Acikgoz et al. (1986) preserved a bone flap between the abdominal fat and muscle. These methods require additional incisions, whereas the preservation of bone flaps under the scalp does not (Korfali and Askoby 1988; Pasaoglu et al. 1996).

Extracorporeal preservation of bone flaps involves their storage in some type of solution, such as 80% ethanol (Kurokawa et al. 1995) or 10% formaldehyde solution (Yamashita et al. 1992). Although no major complications have been observed using these methods, freezing has become the most widely used technique for bone flap preservation. The first cases of cranioplasty using deep-freeze preserved autologous bone flaps were reported in the 1950s (Abbot 1953; Elliott and Scott 1951; Odom et al. 1952). Subsequent histological studies of bone flaps that had been preserved at temperatures between –17°C and –80°C for 7 days to 10 years revealed some histological changes (Asano et al. 1993; Itoh 1991; Osawa et al. 1990; Prolo et al. 1979). Prolo et al. (1979) noted the appearance of various numbers of osteocytes in bone flaps that had been preserved for up to 35 months; the number of osteocytes was not correlated with either storage time or patient age. Itoh (1991) identified osteocytes in bone flaps that had been preserved for periods of not more than 2 years. However, Haversian systems and structural proteins necessary for revitalization of the bone flap remained intact, regardless of the duration of deep-freeze preservation (Assano et al. 1993; Itoh 1991; Osawa et al. 1990; Prolo et al. 1979).

Resorption is the most troubling complication after cranioplasty using deep-freeze preserved autologous bone flaps. Autoclaving of the bone flap (Hancock 1963), the presence of multiple fractures in the flap (Ozaki 1994), the age of the patient (Hancock 1963; Ozaki 1994; Prolo et al. 1979), and shunt operations (Assano et al 1993) have been suggested to be factors relevant in eventual resorption.

Revitalization and remodeling of free bone flaps play complex roles in resorption followed by accretion (Prolo et al. 1979). This ''creeping substitution'' (Phemister 1914) is thought to be initiated by revascularization. Histological studies of replaced free bone flaps yielded evidence of vascularity and the presence of resorption cavities filled by primitive mesenchymal tissue and lined by osteoclasts (Prolo et al. 1979). Revascularization of and osteoblast infiltration into the bone flaps occur from the adjacent bone edge. Therefore, to achieve revitalization and obtain good results after delayed cranioplasty, excellent contiguity between the edge of the bone defect and the autologous bone flap seems to be the most important factor (Prolo et al. 1979).

Infection of the flap is another major complication of delayed cranioplasty using frozen bone flaps (Itoh 1991; Odom et al. 1952). To avoid infection, Osawa et al. (1990) recommended that bone flaps be autoclaved before reimplantation. However, autoclaving has been reported to be a risk for flap resorption (Abbott 1953; Assano et al. 1993; Hancock 1963; Odom et al. 1952; Ozaki 1994). Based on these considerations, we suggest that autologous frozen bone flaps be autoclaved only if infection of the bone flap is suspected during the initial operation or in case the flap might have been infiltrated by tumor cells (Vanaclocha et al. 1997).

Acknowledgements The authors would like to acknowledge the help of Ms. Stavit Kariaff for handling the patient's data.

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