Journal of Clinical Neuroscience 42 (2017) 81–83

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical commentary

Cryostored autologous skull bone for cranioplasty? A study on cranial bone flaps' viability and microbial contamination after deep-frozen storage at −80 °C

David Yuen Chung Chan [a], Yi Tan Mok [b], Ping Kuen Lam [c], Cindy See Wai Tong [c], Stephanie Chi Ping Ng [a], Tin Fung David Sun [a], Wai Sang Poon [a,c,*]

[a] Division of Neurosurgery, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong
[b] Operating Theatre, Prince of Wales Hospital, Hong Kong
[c] Chow Tai Fook – Cheung Yu Tung Surgical Stem Cell Research Centre, The Chinese University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 15 February 2017
Accepted 3 April 2017

Keywords:
Craniectomy
Cryostored
Autologous
Cranial bone flaps
Cranioplasty

ABSTRACT

Craniectomy is a life-saving procedure. Subsequent cranioplasty with autologous skull bone has a bone resorption rate from 4% to 22.8% and an infection rate from 3.3% to 26%. There are concerns with their viability and the potential microbial contamination as they were explanted for a long period of time. Eighteen cranial bone flaps stored at Prince of Wales Hospital Skull Bone Bank during the period from June 2011 to March 2016 were identified. Ethics approval was obtained. Bone chips and deep bone swabs were collected for osteoblast culture and microbial culture. Skull Bone Bank was kept at −80 °C under strict aseptic technique during the study period. The storage period ranged from 4 months to 55 months. For the osteoblast culture, all eighteen bone flaps had no viable osteoblast growth. For the bacterial culture, five had positive bacteria growth (27.8%). Three were *Pasteurella multocida* and two were Methicillin-resistant *Staphylococcus aureus*. The mean duration of storage of the infected bone flap was 32.9 months (±15.1 months) versus 19.9 months (±17.9 months) of those bone flaps with no bacterial growth ($p = 0.1716$). The mean size of the infected versus non-infected bone flaps was 117.7 cm^2 (±44.96 cm^2) versus 76.8 cm^2 (±50.24 cm^2) respectively ($p = 0.1318$). Although in this study statistical significance was not reached, it was postulated that infected bone flaps tended to be larger in size and had a longer duration of storage. In conclusion, cryostored skull bone flaps beyond four months showed no viable osteoblasts. Bacterial contamination rate of bone flaps was 27.8% in this study.

1. Introduction

Craniectomy is a life-saving procedure [1,2]. Subsequent cranioplasty with autologous skull bone has a bone resorption rate from 4% to 22.8% [3,4] and an infection rate from 3.3% to 26% [5–7]. There are concerns with their viability and the potential microbial contamination as they were explanted for a long period of time. Currently there is no standard method to handle the explanted craniectomy bone flaps. A traditional method is to place the bone flap in a subcutaneous pocket at the abdominal wall till retrieval for cranioplasty [8,9]. Alternatively, craniectomy bone flaps can be stored at a hospital Skull Bone Bank in a freezer at −80 °C (with an acceptable range of −70 °C to −90 °C) [10] under aseptic technique. For our current practice, cranial bone flaps were defrosted upon cranioplasty and sterilized according to a standard protocol for autologous tissue [11]. Bone flaps beyond 6 months of storage would be disposed and synthetic materials such as Titanium or acrylic would be used instead. However, there is no strong evidence or large studies from the literature to justify these rationales.

The aim of the study is to generate data on the viability and status of microbial contamination of deep freeze-stored skull bone flaps.

2. Material and methods

Cranial bone flaps stored at Prince of Wales Hospital Skull Bone Bank during the period from June 2011 to March 2016 were iden-

* Corresponding author at: Division of Neurosurgery, Department of Surgery, 4/F Lui Che Woo Clinical Sciences Building, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong. Fax: +852 2637 7974.
E-mail addresses: davidchan@surgery.cuhk.edu.hk (D.Y.C. Chan), myt372@ha.org.hk (Y.T. Mok), pklam@surgery.cuhk.edu.hk (P.K. Lam), cindytong@surgery.cuhk.edu.hk (C.S.W. Tong), stephanieng@surgery.cuhk.edu.hk (S.C.P. Ng), davidsun@surgery.cuhk.edu.hk (T.F.D. Sun), wpoon@cuhk.edu.hk (W.S. Poon).

http://dx.doi.org/10.1016/j.jocn.2017.04.016
0967-5868/

tified to be disposed. Inclusion criteria included bone flaps of those patients either already had synthetic materials for cranioplasty or deceased patients who had craniectomy. The Skull Bone Bank is a deep freezer with multiple doors and separate compartments to maintain the content at the targeted temperature. It was kept at −80 °C (with an acceptable range from −75 °C to −80 °C) under strict aseptic technique. The bone flaps were individually packed with two layers of sterile plastic bags. Before the specimen collection, the bone flaps were taken out from the deep freezer and allowed to thaw for two to four hours to room temperature. The bone specimens collection were conducted in the operating theatre with laminar flow. The operator was scrubbed up and collected the specimens in strict aseptic technique. Bone chips and deep bone swabs and were collected for osteoblasts culture and microbial culture. Sterile bone rongeurs were used to collect bone chips specimens at the core of the bone flaps. The edges of the bone flaps were avoided as the heat energy from the high-speed drill during craniectomy could have affected the osteoblasts' viability. Swabs were also taken at the deep part of the bone flaps to assess the status of microbial contamination at the core of the bone flaps. The sizes of the bone flaps were also measured. The bone flaps' corresponding background demographic of the patients including sex, age, type of operations, data of the operations, comorbidities, explanted duration of the bone flaps were collected. For the osteoblasts culture, the bone chips were collected in sterile phosphate buffer saline (PBS). The washed bone fragments were cultured in 10 mL of Dulbecco's Modified Eagle's medium (DMEM) enriched with 10% fetal bovine serum and incubated at 37 °C in 5% carbon dioxide. Microscopic examination for the outgrowth of cells from the bone fragments were performed at 7, 14 and 21 days. For microbial culture, the deep bone swabs were inoculated onto chocolate agar plate and incubated for 18 h at 37 °C in room air. A further incubation of 48 h would be performed when there was no growth. Standard antibiotic sensitivities were also performed. Ethics approval was obtained from the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethic Committee.

3. Results

During the study period, 18 pieces of skull bone which fulfilled the inclusion criteria were identified. Records confirmed the Skull Bone Bank was kept at −75 °C to −80 °C under strict aseptic technique during the study period. The storage period of the bone flaps ranged from 4 months to 55 months. The background demographic, types of operations, past medical history of the patients were as shown in Table 1. For the osteoblast culture, all 18 had no viable osteoblast growth at 21 days. As a control, one fresh skull bone chip specimen was collected intraoperatively showing positive osteoblasts outgrowth on Day 7 as confirmed by alkaline phosphatase staining. For the bacterial culture, 5 out of 18 had positive bacteria growth (27.8%). Three were *Pasteurella multocida* and two were Methicillin-resistant *Staphylococcus aureus*. All craniectomy were performed as emergency operations. The mean duration of storage of infected versus non-infected bone flap was 32.9 months (±15.1 months) vs 19.9 months (±17.9 months) ($p = 0.1716$). The mean size of the infected versus non-infected bone flap was 117.7 cm^2 (±44.96 cm^2) vs 76.8 cm^2 (±50.24 cm^2) ($p = 0.1318$).

4. Discussion

Cranioplasty has potential complications including infection and bone resorption. Readmission as a result of severe complications from autologous cranioplasty was up to 30% [12]. Bone resorption was up to 22.8% [4] and it was proposed that "an initial allograft should be considered" in selected group of patients [4]. Previous study shown microbial contamination rate of cryostored autologous skull bone flaps at −20 °C was 20% [13]. One study had shown 50% positive culture bacterial growth on the surface of the bone flaps by flora before storage [14]. Another study had reported that cryopreservation of skull bone flap at −30 °C for more than six months shown no viable osteoblasts [15]. On the other hand, 86% of the subcutaneously preserved autologous bone flaps had the presence of osteoblasts [16]. Positive bacterial culture for subcutaneously-stored bone flaps was only one out of fifty [16]. Sterilization with povidone iodine solution, hydrogen peroxide solution and normal saline mixed with antibiotics for autologous bone flap stored at −26 °C had shown a complication rate of 3.40% [17]. On the other hand, sterilization by autoclave was significantly associated with surgical site infection with an infection rate up to 38.5% for bone flap stored at −70 °C [18].

Our current study had shown that cryostored bone flaps had a significant rate of microbial contamination at 27.8% at the deep part of the bone flaps. Further studies would be justified to evaluate the effectiveness of the current sterilization method by soaking the bone flaps into the various antiseptic solution. From the observation of the bone flaps in this current study, the high-speed-drill-treated bone edges of the bone flaps were not completely porous and there were concerns if the antiseptic solutions can penetrate into the deep core part of the bone flaps which yield positive cultures.

As for the lack of viable osteoblasts, this study concurs with the hypothesis that cranioplasty bone flaps could be acting as an "osteoconductive" agent which provide a scaffolding for the osteoblasts from the surrounding via skull bones of the recipients to grow.

Table 1
The results showing no significance in the baseline demographic, types of operations and past medical history of the patients with craniectomy in this study. Though statistically insignificant, infected bone flaps tended to be larger in size and had a longer duration of storage. MRSA: Methicillin-resistant *Staphylococcus aureus*.

		Infected bone flaps	Bone flaps with no growth	*p* Value
Baseline Demographic	Age	65 (55–73)	54 (26–74)	$p = 0.098$
	Gender: Women	0/5 (0%)	2/13 (15.4%)	$p = 0.594$
Types of Operation	Trauma	2/5 (40%)	4/13 (30.8%)	$p = 0.711$
	Emergency	5/5 (100%)	13/13 (100%)	$p = 0.792$
Past medical history	Hypertension	2/5	9/13	$p = 0.266$
	Diabetes	0/5	2/13	$p = 0.594$
	Impaired renal function	1/5	1/13	$p = 0.472$
	Impaired liver function	1/5	2/13	$p = 0.814$
Characteristics of the bone flaps	Size	117.7 cm^2 (±44.96 cm^2)	76.8 cm^2 (±50.24 cm^2)	$p = 0.132$
	Duration of storage	32.9 months (±15.1 months)	19.9 months (±17.9 months)	$p = 0.172$
Types of micro-organisms growth	MRSA	2/5 (40%)	No growth	
	Pasteurella multocida	3/5 (60%)	No growth	

Although in this study statistical significance was not reached, it is postulated that infected bone flaps tend to be larger in size and had a longer duration of storage. Future studies with larger sample size are needed.

5. Conclusions

In this study, cryostored skull bone flaps beyond four months showed no viable osteoblasts. Bacterial contamination rate of bone flaps was 27.8% in this study.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article. There is no financial disclosure.

I confirm that this research meets the ethical guidelines, including adherence to the legal requirements of the study country.

Previous presentation

Part of this paper was previously presented as a poster presentation on 26 November 2016 at the 23rd Annual Scientific Meeting of the Hong Kong Neurosurgical Society in Hong Kong.

Sources of financial support

Nil.

Authors' contributions

DYC, TFS and WSP were the lead investigators and contributed to conception and design of the study. DYC, YTM, PKL, CST and SCN contributed to conduct of the study including collection and process of the specimens. DYC and PKL contributed in data collection. DYC, TFS, SCN and WSP contributed in data analysis and interpretation of the writing of the paper. All authors contributed in revising the paper critically for important intellectual content and final appraisal for publication.

Acknowledgements

We thank the residents at Prince of Wales Hospital for their contribution in the recruitment of patients and acquisition of data.

References

[1] Hutchinson PJ, Kolias AG, Timofeev IS, et al. Trial of decompressive craniectomy for traumatic intracranial hypertension. N Engl J Med 2016;375:1119–30.
[2] Vahedi K, Hofmeijer J, Juettler E, et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. Lancet Neurol 2007;6:215–22.
[3] Schuss P, Vatter H, Oszvald A, et al. Bone flap resorption: risk factors for the development of a long-term complication following cranioplasty after decompressive craniectomy. J Neurotrauma 2013;30:91–5.
[4] Dünisch P, Walter J, Sakr Y, et al. Risk factors of aseptic bone resorption: a study after autologous bone flap reinsertion due to decompressive craniotomy. J Neurosurg 2013;118:1141–7.
[5] Hall WA. Cranioplasty infections – adding insult to injury. World Neurosurg 2014;82:E435–7.
[6] Gooch MR, Gin GE, Kenning TJ, et al. Complications of cranioplasty following decompressive craniectomy: analysis of 62 cases. Neurosurg Focus 2009;26:E9.
[7] Sundseth J, Sundseth A, Berg-Johnsen J, et al. Cranioplasty with autologous cryopreserved bone after decompressive craniectomy: complications and risk factors for developing surgical site infection. Acta Neurochir (Wien) 2014;156:805–11 [discussion 811].
[8] Baldo S, Tacconi L. Effectiveness and safety of subcutaneous abdominal preservation of autologous bone flap after decompressive craniectomy: a prospective pilot study. World Neurosurg 2010;73:552–6.
[9] Shoakazemi A, Flannery T, McConnell RS. Long-term outcome of subcutaneously preserved autologous cranioplasty. Neurosurgery 2009;65:505–10 [discussion 510].
[10] Zwitser EW, Jiya TU, George Licher H, et al. Design and management of an orthopaedic bone bank in The Netherlands. Cell Tissue Bank 2012;13:63–9.
[11] Van Wicklin S, Brubaker S. R C. Guideline for autologous tissue management. Assoc Perioper Regist Nurses 2014:187–238.
[12] El Ghoul W, Harrisson S, Belli A. Autologous cranioplasty following decompressive craniectomy in the trauma setting. Br J Neurosurg 2014;1–6.
[13] Bhaskar IP, Inglis TJJ, Bowman J, et al. Microbial contamination assessment of cryostored autogenous cranial bone flaps: should bone biopsies or swabs be performed? Acta Neurochir (Wien) 2013;155:367–71.
[14] Chiang H, Steelman VM, Pottinger JM, et al. Clinical significance of positive cranial bone flap cultures and associated risk of surgical site infection after craniotomies or craniectomies. J Neurosurg 2011;114:1746–54.
[15] Bhaskar IP, Yusheng L, Zheng M, et al. Autogenous skull flaps stored frozen for more than 6 months: do they remain viable? J Clin Neurosci 2011;18:1690–3.
[16] Singla N, Parkinson Singh S, Gupta SK, et al. Histopathology of subcutaneously preserved autologous bone flap after decompressive craniectomy: a prospective study. Acta Neurochir (Wien) 2014;156:1369–73.
[17] Tahir MZ, Shamim MS, Sobani ZA, et al. Safety of untreated autologous cranioplasty after extracorporeal storage at −26 degrees Celsius. Br J Neurosurg 2013;27:479–82.
[18] Wui S-H, Kim KM, Ryu Y-J, et al. The Autoclaving of Autologous Bone is a Risk Factor for Surgical Site Infection After Cranioplasty. World Neurosurg 2016;91:43–9.