Analyses Using Micro-CT Scans and Tissue Staining on New Bone Formation and Bone Fusion According to the Timing of Cranioplasty via Frozen Autologous Bone Flaps in Rabbits: A Preliminary Report

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Objective: The timing of cranioplasty and method of bone flap storage are known risk factors of non-union and resorption of bone flaps. In this animal experimental study, we evaluated the efficacy of cranioplasty using frozen autologous bone flap, and examined whether the timing of cranioplasty after craniectomy affects bone fusion and new bone formation.

Methods: Total 8 rabbits (male, older than 16 weeks) were divided into two groups of early cranioplasty group (EG, 4 rabbits) and delayed cranioplasty group (DG, 4 rabbits). The rabbits of each group were performed cranioplasty via frozen autologous bone flaps 4 weeks (EG) and 8 weeks (DG) after craniectomy. In order to obtain control data, the cranioplasty immediate after craniectomy were made on the contralateral cranial bone of the rabbits (control group, CG). The bone fusion and new bone formation were evaluated by micro-CT scan and histological examination 8 weeks after cranioplasty on both groups.

Results: In the micro-CT scans, the mean values of the volume and the surface of new bone were 50.13±7.18 mm³ and 706.23±77.26 mm² in EG, 53.78±10.86 mm³ and 726.60±170.99 mm² in DG, and 31.51±12.84 mm³ and 436.65±132.24 mm² in CG. In the statistical results, significant differences were shown between EG and CG and between DG and CG (volume: p=0.028 and surface: p=0.008). The histological results confirmed new bone formation in all rabbits.

Conclusion: We observed new bone formation on all the frozen autologous bone flaps that was stored within 8 weeks. The timing of cranioplasty may showed no difference of degree of new bone formation. Not only the healing period after cranioplasty but the time interval from craniectomy to cranioplasty could affect the new bone formation.

Key Words: Cranioplasty · Timing · Frozen stored · Autologous bone · Rabbits.

INTRODUCTION

Decompressive craniectomy is very effective method of reducing intracranial pressure. In a recent prospective clinical trial, decompressive craniectomy was confirmed to have reduced the mortality by 50% in the patients with cerebral edema caused by cerebral infarction12-14,43). In addition, it was effective for cerebral edema caused by traumatic brain damage29), subarachnoid hemorrhage30,31), intracerebral hemorrhage32), and cranial venous and sinus thromboses33). Cranioplasty is performed after craniectomy when intracranial pressure is under control for functional and aesthetic restorations. During the cranioplasty procedure, autologous bone flaps are preferably used due to their advantages in storage, viability, cost, prevention of disease transmission, and aesthetics. However, cranioplasty that uses the autologous bone flap has a risk of developing complications such as infection, intracranial hemorrhage, seizure, and hydrocephalus. Long-term complications, such as bone flap resorption and bone non-union and resorption of bone flaps. In this animal experimental study, we evaluated the efficacy of cranioplasty using frozen autologous bone flap, and examined whether the timing of cranioplasty after craniectomy affects bone fusion and new bone formation.
union, have incidence rates that reached 2–17%\(^{18,21,33}\). According to the studies on human cranioplasty, the timing of performing cranioplasty is one of the critical factors that may develop autologous bone flap resorption and bone non-union\(^{19}\). Although generally accepted concept about timing of cranioplasty using autologous bone is that early cranioplasty has more risk of infection and delayed cranioplasty has risk of non-union or resorption of bone flap\(^{16,20,40}\), the debates are still remained. Another report showed non-union and/or resorption of bone flap occurred more frequently on early cranioplasty patients who had surgery-related infections\(^{40}\). And the other said there were no relation of bone non-union to timing of cranioplasty\(^{40}\).

In this study, cranioplasty was performed on rabbits using frozen autologous bone flaps, while the levels of new bone formation and bone fusion after craniectomy were observed, according to the timing of cranioplasty to evaluate its efficacy.

**MATERIALS AND METHODS**

**Experimental animals and groups**

Experiments were conducted in compliance with the animal experimentation ethics upon approval from the Institutional Animal Care and Use Committee of our institute. Eight adult (16 weeks or older) male New Zealand white rabbits with the body weights ranging from 3.2 kg to 4.2 kg were used in this study. Four out of eight subjects were assigned to the delayed cranioplasty group (DG), and the remaining four subjects were assigned to the early cranioplasty group (EG). At day 0, craniotomy was performed at the right frontoparietal bone of the DG rabbit, and the obtained bone flap was stored in a freezer at a temperature of -80°C. At day 28 or week 4, bone defect was made in the right frontoparietal bone of the EG rabbit, and then, the bone flap was stored at the same condition. At day 56 or week 8, the frozen bone flap was fixed on the bone defect area of the DG and EG rabbits. In order to obtain control data, the same procedures were made on the left cranial bone of the rabbits, and the bone flap was immediately fixed (control group, CG). At day 112 or week 16, the EG and DG rabbits were sacrificed to conduct radiological and histological tests on their cranial bones (Fig. 1).

The time span between craniectomy and cranioplasty was the healing time of bone defect and defined as “the primary healing time”, while the time between the cranioplasty and rabbit sacrifice was the bone formation time of the gap between bone flap and cranium and defined as “the secondary healing time”.

**Preparation of the experimental model**

**Formation of bone defect and storage of bone flap**

In order to induce anesthesia on the rabbits, 0.5 mL/kg of ketamine HCl (Ketalar, Yuhan Corporation, Seoul, Korea) and 0.25 mg/kg of xylazine HCl (Rompun, Bayer, Pittsburgh, PA, USA) were mixed and injected intramuscularly. For local anesthesia and hemorrhage control, 2% lidocaine HCl (Yuhan Corporation, Seoul, Korea) containing 1 : 100000 epinephrine was injected subcutaneously. After a 4-cm long incision was made along the midline of the cranial skin, the muscles and peristeum were incised layer by layer to expose the cranial bone (Fig. 2A). In order to make the bone flap, a bone flap margin was formed 3 mm lateral to the sagittal suture, which was located at the right side for the experimental groups and the left side for the control group, using a trephine drill with a 10-mm outer diameter and 9-mm inner diameter. To avoid damaging the dura mater, the inner table of the cortical bone was left (Fig. 2B, C).

Using a 1-mm round burr, the space between the round bone flap margin and the cranial bone was expanded out of the bone flap margin. Using a 1-mm round burr, the space between the round bone flap margin and the cranial bone was expanded out of the bone flap margin.
Cranioplasty

After anesthesia and skin incision that mentioned above, the fibrous tissues, which were formed during the primary healing time, were removed using a curette in order to expose the bone defect area (Fig. 3A). Afterwards, the autologous bone flap, which has been kept in the freezer, was applied to each labeled rabbit in order to perform cranioplasty. The cranial bone and the bone flap were fixed using a titanium-alloy miniplate and 3-mm screws (Synthes Inc., West Chester, PA, USA) (Fig. 3B, C). Using the same method, CG was formed on the left cranium of the rabbit (Fig. 3D).

Radiologic observation

At week 16, the cranial bone of the sacrificed rabbit was scanned by the micro-computed tomography (micro-CT) (SkyScan 1173, SKYSCAN, Kontich, Belgium). The 2240×2240 pixel sectional images (Fig. 4A, B) and the three dimensional reconstructed images (Nrecon reconstruction program, SKYSCAN, Kontich, Belgium) (Fig. 4C, D) were obtained. After confirming the round bone flap through the micro-CT images and 3D remodeling images, we defined the region of interest (ROI) as the region within a 14-mm diameter from center point of bone flap. We measured the bone volume (VOLROI) and the bone surface (SURROI) of ROI (Fig. 5A). From VOLROI and SURROI, the bone volume (VOLOB) and the bone surface (SURBO) of the bone flap, and the volume (VOLmetal) and the surface (SURmetal) of metal fixture were removed. Afterwards, the CT scan images (Fig. 5B) and the 3D reconstruction images (Fig. 5C) of the new bone volume (VOLnew) and the new bone surface (SURnew) were quantified and measured.

\[ VOL_{\text{new}} = VOL_{\text{ROI}} - (VOL_{\text{bone flap}} + VOL_{\text{metal}}) \]
tion, and none of them dropped out of the experiments conducted in this study. New bone formation was confirmed in all groups. The new bone formation observed in the micro-CT images was represented as bone volume (VOL\textsubscript{new}) and bone surface (SUR\textsubscript{new}).

### Table 1. New Bone Formation in the Micro-CT Images

| Rabbit ID | Group     | VOL\textsubscript{new} (mm\(^3\)) | SUR\textsubscript{new} (mm\(^2\)) |
|-----------|-----------|-----------------------------------|----------------------------------|
| E1        | Early     | 40.97                             | 604.69                           |
|           | Control   | 15.99                             | 332.59                           |
| E2        | Early     | 49.64                             | 707.72                           |
|           | Control   | 16.07                             | 236.85                           |
| E3        | Early     | 49.65                             | 701.25                           |
|           | Control   | 30.90                             | 447.70                           |
| E4        | Early     | 58.42                             | 792.25                           |
|           | Control   | 35.46                             | 557.93                           |
| D1        | Delayed   | 68.00                             | 970.33                           |
|           | Control   | 36.35                             | 563.11                           |
| D2        | Delayed   | 43.93                             | 619.41                           |
|           | Control   | 30.02                             | 378.78                           |
| D3        | Delayed   | 48.35                             | 597.10                           |
|           | Control   | 56.54                             | 613.55                           |
| D4        | Delayed   | 55.85                             | 719.56                           |
|           | Control   | 32.63                             | 451.90                           |

VOL\textsubscript{new}: bone volume of new bone, SUR\textsubscript{new}: bone surface of new bone, CT: computed tomography.
face (SUR\textsubscript{new}) (Table 1). The mean new bone volume of the DG was 53.78±10.86 mm\(^3\), while the mean bone surface was 726.60±170.99 mm\(^2\). The mean new bone volume and mean bone surface of the EG were 30.13±7.18 mm\(^3\) and 706.23±77.26 mm\(^2\), respectively. The mean new bone volume of the CG was 31.51±12.84 mm\(^3\), while the mean bone surface was 436.65±132.24 mm\(^2\). Statistically significant differences were observed in the mean new bone volume (p=0.024) and bone surface (p=0.008) among the three groups (Table 2). In the post hoc test, no statistically significant difference in bone volume (p=0.886) and bone surface (p=0.886) was observed between the DG and EG. When the CG was compared with the EG and DG, significant differences in bone volume (p=0.028) and bone surface (p=0.008) were observed in both comparisons (Table 3).

Similar to the findings in the micro-CT images, new bone formation was observed in the microscopic examination of the H-E stain and Goldner’s stain in all of the rabbit tissues. The new bone formation was observed as a form of 1) a bony islet between the bone flap and the edge of the cranial bone (Fig. 6A), 2) a bony islet at the lower part of the bone flap (Fig. 6B), 3) a new bone formation at the edge of the cranial bone (Fig. 6C), and 4) a bone flap incorporation between the bone flap and the edge of the cranial bone (Fig. 6D).

### DISCUSSION

Cranioplasty, which is performed after craniectomy, has aesthetic and functional advantages, and it is used in a wide variety of diseases. However, various complications such as infection, intracranial hemorrhage, bone flap resorption, depressed bone flap, cerebral vasospasm, and hydrocephalus can develop after the cranioplasty procedure, and their incidence rate has been reported to be 15–36.5%\(^{4,5,8,14,20,27,32,36}\). The materials that are most frequently used in cranioplasty include autologous bone flap, poly-methyl-methacrylate (PMMA), and hydroxyapatite. The PMMA is known to show a similar frequency of complication development to that of the autologous bone flap, but its osteogenesis capability is much less than that of the autologous bone flap\(^{22,24}\). Hydroxyapatite can supplement the low osteogenesis capability of PMMA; however, it is expensive\(^{39}\). The autologous bone flap is the most widely used cranioplasty material due to its superiority in the viability, cost, prevention of disease transmission, and aesthetics to artificial bones. Nevertheless, long-term complications such as bone non-union and resorption, which develop after the cranioplasty procedure using the autologous bone flap, are the major causes that require re-operation.

In a cranioplasty using autologous bone flap, bone flap incorporation occurs in the process of revascularization, osteo-

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**Table 2.** The mean values of bone volume (VOL\textsubscript{new}) and bone surface (SUR\textsubscript{new}) of new bone in the micro-CT images*

| Group            | VOL\textsubscript{new} (mm\(^3\), mean±SD) | SUR\textsubscript{new} (mm\(^2\), mean±SD) |
|------------------|-------------------------------------------|-------------------------------------------|
| Early group      | 50.13±7.182                               | 706.23±77.26                              |
| Delayed group    | 53.78±10.86                               | 726.60±170.99                             |
| Control          | 31.51±12.84                               | 436.65±132.24                             |

*As a non-parametric test, Kruskal-Wallis test was used for the comparison among three groups. Statistically significant differences were observed in the mean new bone volume (p=0.024) and bone surface (p=0.007) among the three groups.

**Table 3.** The post hoc test of each paired groups*

|                  | VOL\textsubscript{new} (mm\(^3\), mean±SD) | SUR\textsubscript{new} (mm\(^2\), mean±SD) | Sig. (p) |
|------------------|-------------------------------------------|-------------------------------------------|----------|
| Early group vs. delayed group |                                           |                                           |          |
| Early group      | 50.13±7.182                               | 706.23±77.26                              | 0.886 (BV) |
| Delayed group    | 53.78±10.86                               | 726.60±170.99                             | 0.886 (BS) |
| Early group vs. control |                                           |                                           |          |
| Early group      | 50.13±7.182                               | 706.23±77.26                              | 0.028 (BV) |
| Control          | 31.51±12.84                               | 436.65±132.24                             | 0.008 (BS) |
| Delayed group vs. control |                                           |                                           |          |
| Delayed group    | 53.78±10.86                               | 726.60±170.99                             | 0.028 (BV) |
| Control          | 31.51±12.84                               | 436.65±132.24                             | 0.008 (BS) |

*As a post hoc test, Mann-Whitney test was used. No significant difference in bone volume and bone surface of new bone was observed between the delayed and the early group. When the control group was compared with the delayed and the early group, significant differences in bone volume and bone surface were observed in both comparisons. BV: bone volume, BS: bone surface, SD: standard deviation, VOL\textsubscript{new}: bone volume of new bone, SUR\textsubscript{new}: bone surface of new bone.

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**Fig. 6.** The histological patterns of new bone formation. The new bone formation was observed in the microscopic examination of the hematoxylin and eosin stain and Goldner’s stain in all of the rabbit tissues. The new bone formation was observed as a form of 1) a bony islet (arrow) between the bone flap and the edge of the cranial bone (A), 2) a bony islet (arrow) at the lower part of the bone flap (B), 3) a new bone formation (arrow) at the edge of the cranial bone (C), and 4) a bone flap incorporation (arrow) between the bone flap and the edge of the cranial bone (D).
conduction, resorption, and osteogenesis. In a bone union process, bone remodeling is completed through the repetition of bone resorption and new bone formation. Bone resorption is known to happen when problems arise during the bone union process. The patient-side risk factors of bone resorption are known to include young age, traumatic cranial damage, multiple cranial fractures, and the size of the cranial defect. Meanwhile, iatrogenic factors, which include the timing of cranioplasty, have been reported.

According to previous studies conducted on the timing of cranioplasty, its early performance resulted in an increase in the incidence of infection, while delayed performance resulted in an increase in the resorption of the autologous bone flap. However, Piedra et al. reported that there is no difference in the frequency of the development of complications, such as bone non-union and resorption, and in the infection between the early and delayed cranioplasty performance groups. In the case of cranioplasty performed within two months after craniectomy, Schuss et al. reported a high frequency of infection, and resultant bone non-union and resorption. In this study, the timing of cranioplasty was set at 4 and 8 weeks with the EG and DG, and no significant difference in new bone formation was observed between the groups. Furthermore, when the frozen autologous bone flap was used on rabbits within 8 weeks, an active new bone formation was radiologically and histologically confirmed, and no significant difference in new bone formation was observed according to the freezing period.

There are several methods of storing the autologous cranial bone during the period between the procedures of craniectomy and cranioplasty. The method of freezing the autologous bone flap was introduced in the 1950s, and since then, it has been the most widely used method. The most important factor to consider for a successful bone flap incorporation after cranioplasty is the viability of the bone flap. When the frozen autologous bone flap was histologically analyzed at temperatures between -17°C and -80°C, the Harvesian system of the bone tissues and structural proteins were confirmed to have remained the same regardless of the freezing period, and the osteocytes were observed even until the 35th month after freezing. Moreover, the freezing storage method is superior to the other methods in terms of preserving the cellular architecture. An example of this is the cytoplasm of the lacunar cells, which was well preserved. In the frozen cranial bone, new blood vessels are remodeled, and they play the role of an architectural frame that assists the growth of the osteoprogenitor cells. In addition, revascularization and infiltration of the osteoblasts occur from the edge of the cranial bone toward the bone flap. These phenomena were also confirmed in this study. A new bone formation was observed at the edge of the cranial bone close to the bone flaps of all the DG and EG rabbits not only in the micro-CT, but also in the histological results. A bony islet formation was also observed. According to Sultan et al., the bony islet formation contributed to the promotion of bone flap incorporation and the reduction of bone flap resorption.

When cranioplasty is performed immediately after craniectomy, the best bone flap viability and a favorable new bone formation are usually observed. In this study, however, the CG’s new bone formation was significantly the worst when compared with the EG and DG. This may be due to the difference in the total healing time—primary healing time in the CG, 8 weeks in the DG and 4 weeks in the EG—even though the three groups had the same secondary healing time of 8 weeks. According to Sohn et al., osteogenesis is a consistent process, but the time of initiating bone marrow maturity and bony islet formation in rabbits after the craniectomy procedure was 8 weeks after the surgery. This means that it takes 8 to 12 weeks to clearly observe and assess bone flap incorporation, bone remodeling, and osteogenesis. In this study, early bone flap incorporation was assessed through the early assessment on the healing process of the defect area. The primary healing time of the EG and DG was 4 weeks and 8 weeks, respectively, and their total healing time was 12 weeks and 16 weeks, respectively, while the total healing time of the CG was 8 weeks. This implies that the time of initiating new bone formation and bone flap incorporation is affected not only by the healing time after cranioplasty (secondary healing time), but also by the total period after craniectomy (primary and secondary healing time). Accordingly, the primary healing time given prior to bone transplantation may positively affect the bone healing process. The bone remodeling period after fracture on rabbits is widely known as 6–8 weeks of period—1 week of resorption, 0.5–1 week of reversal and 4.5–6 weeks of bone formation (on human, total 17 weeks–2 weeks of resorption, 2 weeks of reversal and 13 weeks of bone formation). At this point of view, the cranioplasty performing before end of bone remodeling period would have some advantages to obtain bone fusion. Thus, we think that cranioplasty performed within 8 weeks of primary healing time could result in good bone fusion.

This study had some limitations. Small sample size and the lack of power analysis for estimating appropriate sample are major concerns of this study. Despite of small sample size, the experimental result using animal models that the period between craniectomy and cranioplasty could affect the bone fusion is a meaningful result of this study. Another limitation is the different periods of total healing time and primary healing time among groups, because the duration of healing time after craniectomy has been known as one of important factors of bone fusion and union. We suggested more precisely designed study that focused on variations of healing duration among the groups should be mandatory. Considering the preliminary characteristic of this study, these limitations are expected to be surmounted on well-designed large study on future.
CONCLUSION

In this study, the cranioplasty procedure conducted on rabbits using the autologous bone flap frozen for less than 8 weeks resulted in favorable new bone formation and bone flap incorporation. These results were radiologically and histologically confirmed. Furthermore, not only the post-cranioplasty healing time (secondary healing time), but also the primary healing time, which was the time between the procedures of craniectomy and cranioplasty, were considered to positively affect the new bone formation and bone flap incorporation processes. Based on the confirmation in this study regarding the positive healing process of rabbits even during the delayed period, the delayed period between the craniectomy and cranioplasty procedures in humans may positively affect the bone flap incorporation process. Further studies on the ideal primary healing time may be required in the future.

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References

1. Abbott KH : Use of frozen cranial bone flaps for autogenous and homologous grafts in cranioplasty and spinal interbody fusion. J Neurosurg 10: 380-388, 1953
2. Ahmadi SA, Meier U, Lernche J : Detailed long-term outcome analysis after decompressive craniectomy for severe traumatic brain injury. Brain Inj 24: 1539-1549, 2010
3. Asano Y, Ryuie Y, Hasuo M, Simosawa S : [Cranioplasty using cryopreserved autogenous bone]. No To Shinkei 45: 1145-1150, 1993
4. Chang V, Hartfield P, Langholt M, Mahmoud A, Seyfried D : Outcomes of cranial repair after craniectomy. J Neurosurg 112: 1120-1124, 2010
5. Chiabraro S, Di Rocco F, Minone G, Fracia M, Maksen O, Di Emidio P, et al. : Decompressive craniectomy and early cranioplasty for the management of severe head injury : a prospective multicenter study at 147 patients. World Neurosurg 75: 558-562, 2011
6. Chun HJ, Yi HJ : Efficacy and safety of early cranioplasty, at least within 1 month. J Craniofac Surg 22: 203-207, 2011
7. Coutinho JM, Majoue CB, Coert RA, Stam J : Decompressive hemicraniectomy in cerebral sinus thrombosis : consecutive case series and review of the literature. Stroke 40: 2233-2235, 2009
8. De Bonis P, Pomppucci A, Mangiola A, D’Alessandris QG, Rigante A, Anle C : Decompressive craniectomy for the treatment of traumatic brain injury : does an age limit exist? J Neurosurg 112: 1150-1153, 2010
9. DeLuca L, Raszewski R, Tresser N, Gayuron B : The fate of preserved autogenous bone graft. Plast Reconstr Surg 99: 1324-1328, 1997
10. Dorfer C, Frick A, Knoop E, Gruber A : Decompressive hemicraniectomy after aneurysmal subarachnoid hemorrhage. World Neurosurg 74: 465-471, 2010
11. Elliott H, Scott HJ : The bone-bank in neurosurgery. Br J Surg 39: 31-34, 1951
12. Elsantaly ME, Genecev DG : Bone grafts in craniofacial surgery. Cranio-Maxillofac Trauma Reconsr 2: 125-134, 2009
13. Frassanito P, Massimi L, Caldarelli M, Tamburrini G, Di Rocco C : Complications of delayed cranial repair after decompressive craniectomy in children less than 1 year old. Acta Neurochir (Wien) 154: 927-933, 2012
14. Gooch MR, Gin GE, Kenning TJ, German JW : Complications of cranioplasty following decompressive craniectomy : analysis of 62 cases. Neurosurg Focus 26: e89, 2009
15. Grant GA, Jolley M, Ellenbogen RG, Roberts TS, Gruss JR, Loesser JD : Failure of autologous bone-assisted cranioplasty following decompressive craniectomy in children and adolescents. J Neurosurg 100 (2 Suppl Pediatrics) : 163-168, 2004
16. Güresir E, Raabe A, Setzer M, Vatter H, Gerlach R, Seifert V, et al. : Decompressive hemicraniectomy in subarachnoid haemorrhage : the influence of infarction, haemorrhage and brain swelling. J Neurol Neurosurg Psychiatry 80: 799-801, 2009
17. Hofmeijer J, Kappelle LJ, Algra A, Amelnik GJ, van Gin J, van der Vorp HB, et al. : Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarction with Life-threatening Edema Trial [HAMLET]) : a multicentre, open, randomised trial. Lancet Neurol 8: 326-333, 2009
18. Honeybul S : Complications of decompressive craniectomy for head injury. J Clin Neurosci 17: 430-435, 2010
19. Hutchinson PJ, Corte K, Csonynka M, Mendelow AD, Monon DK, Mitchell P, et al. : Decompressive craniectomy in traumatic brain injury : the randomized multicenter RESCUEicp study (www.RESCUEicp.com). Acta Neurochir Suppl 96: 17-20, 2006
20. Im SH, Jang DK, Han YM, Kim JI, Chung DS, Park YS : Long-term incidence and predicting factors of cranioplasty infection after decompressive craniectomy. J Korean Neurosurg Soc 52: 396-403, 2012
21. Iwama T, Yamada Y, Isai S, Shinoda J, Funakoshi T, Sakai N : The use of frozen autogenous bone flaps in delayed cranioplasty revisited. Neurosurgery 52: 591-596; discussion 595-596, 2003
22. Jaberi J, Gambrell K, Twana P, Madden C, Finn R : Long-term clinical outcome analysis of poly-methyl-methacrylate cranioplasty for large skull defects. J Oral Maxillofac Surg 71: e81-e88, 2013
23. Jäntner E, Schwab S, Schmedek P, Untereg A, Hennerici M, Woitzak I, et al. : Decompressive Surgery for the Treatment of Malignant Infarction of the Middle Cerebral Artery (DESTINY) : a randomized, controlled trial. Stroke 38: 2518-2525, 2007
24. Klinger DR, Madden C, Beshay J, White J, Gambrell K, Rickert K : Autologous and acryl cranioplasty : a review of 10 years and 258 cases. World Neurosurg 82: e525-e530, 2014
25. Matsumo A, Tanaka H, Iwamura H, Takamash H, Miyawaki S, Nakashima M, et al. : Analyses of the factors influencing bone graft infection after delayed cranioplasty. Acta Neurochir (Wien) 140: 535-540; discussion 540, 2006
26. Misch CE : Implantologia Contemporânea. Madrid : Mosby, 1995, pp324-350
27. Morina A, Kelmendi F, Morina Q, Dragusha S, Ahmeti F, Morina D, et al. : Cranioplasty with subcutaneously preserved autologous bone grafts in abdominal wall-Experience with 75 cases in a post-war country Kosovo. Surg Neurol Int 2: 72, 2011
28. Moruassagi K, Ver Helen J, Ganchi P, Amin-Hanjani S, Mesa J, Yaremchuk M : Cranioplasty with subcutaneously preserved autologous bone grafts. Plast Reconstr Surg 117: 202-206, 2006
29. Murphy JT, Chowday GV, Murphy TV, Bhasha PS, Naryanan TJ : Decompressive craniectomy with clot evacuation in large hemispheric hypertensive intracerebral hemorrhage. Neurocrit Care 2: 258-262, 2005
30. Odom CL, Woodhall B, Wrenn FR : The use of refrigerated autogenous bone flaps for cranioplasty. J Neurosurg 99: 606-610, 1952
31. Osawa M, Hara H, Ichinose Y, Koyama T, Kobayashi S, Sugita Y : Cranioplasty with a frozen and autoclaved bone flap. Acta Neurochir
32. Piedra MP, Ragel BT, Dogan A, Copra ND, Delashaw JB: Timing of cranioplasty after decompressive craniectomy for ischemic or hemorrhagic stroke. *J Neurosurg* 118:109-114, 2013
33. Polin RS, Shaffrey ME, Bogaev CA, Tisdale N, Germanson T, Bocchichio B, et al.: Decompressive bifrontal craniectomy in the treatment of severe refractory posttraumatic cerebral edema. *Neurosurgery* 41:84-92; discussion 92-94, 1997
34. Prolo DJ, Burres KP, McLaughlin WT, Christensen AH: Autogenous skull cranioplasty: fresh and preserved (frozen), with consideration of the cellular response. *Neurosurgery* 4:18-29, 1979
35. Schuss P, Vatter H, Marquardt G, Imolí L, Ulrich CT, Seifert V, et al.: Cranioplasty after decompressive craniectomy: the effect of timing on postoperative complications. *J Neurotrauma* 29:1090-1095, 2012
36. Sobani ZA, Shamim MS, Zafar SN, Qadeer M, Bilal N, Murtaza SG, et al.: Cranioplasty after decompressive craniectomy: an institutional audit and analysis of factors related to complications. *Surg Neurol Int* 2:123, 2011
37. Sohn JY, Park JC, Um YJ, Jung UW, Kim CS, Cho KS, et al.: Spontaneous healing capacity of rabbit cranial defects of various sizes. *J Periodontal Implant Sci* 40:180-187, 2010
38. Stefini R, Esposito G, Zanotti B, Iaccarino C, Fontanella MM, Servadei F: Use of “custom made” porous hydroxyapatite implants for cranioplasty: postoperative analysis of complications in 1549 patients. *Surg Neurol Int* 4:12, 2013
39. Stevenson S, Li XQ, Davy DT, Klein L, Goldberg VM: Critical biological determinants of incorporation of non-vascularized cortical bone grafts. Quantification of a complex process and structure. *J Bone Joint Surg Am* 79:1-16, 1997
40. Stiver SI: Complications of decompressive craniectomy for traumatic brain injury. *Neurosurg Focus* 26:E7, 2009
41. Sultan SM, Davidson EH, Butala P, Schachar JS, Witek L, Szpalski C, et al.: Interval cranioplasty: comparison of current standards. *Plast Reconstr Surg* 127:1855-1864, 2011
42. Vahedi K, Hofmeijer J, Juettler E, Vicaut E, George B, Algra A, et al.: Sequential-design, multicenter, randomized, controlled trial of early decompressive craniectomy in malignant middle cerebral artery infarction (DECIMAL Trial). *Stroke* 38:2506-2517, 2007
43. Vahedi K, Vicaut E, Mateo J, Kurtz A, Orabi M, Guichard JP, et al.: Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. *Lancet Neurol* 6:215-222, 2007
44. Waziri A, Fusco D, Mayer SA, McKhann GM 2nd, Connolly ES Jr: Postoperative hydrocephalus in patients undergoing decompressive hemicraniectomy for ischemic or hemorrhagic stroke. *Neurosurgery* 61:489-493; discussion 493-494, 2007