Promoting ossification of calvarial defects in craniosynostosis surgery by demineralized bone plate and bone dust in different age groups☆

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Summary   Correction of calvarial defects after calvarial vault reconstruction (CVR) is challenging in craniosynostosis patients of advanced age and typically employs autologous bone. Demineralized bone matrix (DBM) is a potential alternative material for autologous bone, but its use has not been extended to correct calvarial defects.

CVR patients operated at the Department of Plastic Surgery, Helsinki University Hospital, during 2008–2010 were retrospectively reviewed. Inclusion criteria of the study were CVR patients who received DBM plate, with or without bone dust, on calvarial defects and who had suitable uncovered defect on the contralateral side as control. This study included 17 craniosynostosis and one positional plagiocephaly patient, whose mean age was 6.9 years (range 0.9–19 years). The mean follow-up time was 5.6 years. The fusion degree of all defects was measured from 1 week to 1 year postoperatively using three-dimensional computed tomography (3D CT) images by the OsiriX© method. Medical records were reviewed for DBM-related complications.

A total of 26 defects were covered with a DBM plate (mean area 11.1 cm^2) and 26 control defects were identified (mean area 7.8 cm^2). The mean fusion degree of the DBM defects was 74% and 54% for the controls ($p < 0.001$). The mean fusion degree of nine DBM defects that lacked bone dust deposition was 66% and 55% for the nine controls ($p < 0.059$). The difference between the DBM and control defects was statistically significant for patients older than 30 months ($p < 0.03$). No DBM-related complication was observed.

DBM plate is a safe and useful material to promote ossification in calvarial defects in CVR. Furthermore, DBM appears to be more effective in older patients ($>$30 months) than in younger patients or when used with bone dust.

Introduction

Calvarial defects are ossified by the osteogenic potential of dura after calvarial vault reconstruction (CVR) up to the age of 24 months.[1,2] Clinical experience has shown that ossification might occur for older patients.[3] Nevertheless, most patients having unossificated calvarial defects are older than 24 months. Correction of these defects remains a challenge. Split calvarial autograft might not be available and other sources of autogenous bone require a second surgery, thereby increasing the operation time and complication risk.[4,5] Donor sites often have problems with long-lasting pain[5,4,6]; however, synthetic materials fail to accompany the growing skull.

These problems have encouraged clinicians to seek alternative materials. The optimal material should be osteoconductive, osteoinductive, easy to handle, and cause minimal inflammatory reaction. It should have a low infection rate, accompany with the growing skull, and be affordable.[6,7] Alternative materials currently used for pediatric patients are calcium carbonate, bioactive glass, and demineralized bone (DBM).[6–9] All these materials have disadvantages such as fragmentation, resorption, infection, and challenging handling properties.[6–8,10,11]

DBM is a potential alternative material. It is prepared from allograft human donor cortical bone by acid extraction of the mineralized component with retention of the extracellular matrix (ECM). ECM is formed from collagen and noncollagenous proteins, including growth factors.[12–14] These ECM particles provide an osteoconductive and osteoinductive scaffold.[12–15] DBM is widely used in orthopedics, but has not been routinely used in the calvarial region. DBM putty has been used with variable results to correct pediatric calvarial defects.[16,17] DBM putty presents challenging handling properties; thus, in order to overcome this, we have used a DBM plate. Literature and clinical experience have shown that bone dust deposition on calvarial defects has positive effect on ossification in CVR.[18] Bone dust, if available, was combined with the DBM.

The aim of this study was to examine the effectiveness and safety of a DBM plate to promote ossification of calvarial defects in craniosynostosis surgery. A further aim was to evaluate the effect of bone dust deposition on ossification and effectiveness of the DBM plate on different age groups.

Materials and methods

A retrospective review of CVR patients operated between 2008 and 2010 was conducted at the Department of Plastic Surgery, Helsinki University Hospital, from 2008 to 2014.

Methods of the retrospective study

Inclusion criteria of the study were CVR patients, who received a DBM plate (DBX strip, Synthes, West Chester, Pa, USA.) on one or more defects, had suitable uncovered defects which acted as controls, and in whom both 1-week and 12-month postoperative three-dimensional computed tomography (3D CT) datasets are available. We have used DBM on craniofacial bony defects for approximately 10 years. We decided to choose CVR patients on the retrospective study for several reasons: this was the largest DBM-treated group, the operation technique was most similar, control defects were available, and the follow-up time was reasonable.

The objective was to select from 3D-CT reconstructions equally sized and periosteum-covered defects from the contralateral side as controls. If a control was unavailable on the contralateral side, the most analogous defect by area was chosen as control. Patient demographical data and DBM-related complications were collected from electronic medical records.

Patients and operations

The patient characteristics are presented in Table 1. A total of 117 patients were reviewed. In this study, 17 craniosynostosis patients and 1 patient with deformational plagiocephaly operated by an experienced craniofacial team were included (Table 1). Their mean age was 6.9 years (SD 5.8, range: 0.8–19) (Table 1). Eight patients received DBM on two defects and thus 26 calvarial defects were covered by the DBM plate. An informed consent to use DBM was received preoperatively from patients' guardians. This material is part of a larger project approved by the Ethical Committee Group of the Helsinki University Hospital.

If large calvarial defects were formed during CVR, one to two calvarial defects were covered by the DBM plate. Because of its high cost (3600€ each), the craniofacial team had only one DBM plate with dimensions of 5 × 5 cm available per surgery. Therefore, some defects were too large or unsuitable in configuration to be covered by such a DBM plate. The DBM plate was deposited on the largest suitable defect, cut analogous to the shape of the defect, and anchored to the surrounding bony framework by PDS 3-0 resorbable sutures. The remnants of the cut plate, if available, were deposited on the suitable shaped defect.

The bone dust left over from craniotomy, if available, was placed on the most critical defect according to the surgeons' clinical evaluation. Bone dust was deposited on 14 defects: nine DBM and five control defects (Table 2). The

Table 1 Patient characteristics.

Patient number	Gender	Age (years)	Diagnosis	Operation	Radiological follow-up (days)	Clinical follow-up (years)	Location of the DBM defect	Location of the control defect
1	M	4.2	Synostosis sagittal suture	CVR	357	6	Left occipital	Right occipital
2	M	8.6	Synostosis sagittal suture	Re-do-CVR	363	5.7	Right anterior parietal and right posterior parietal	Left anterior parietal and left posterior parietal
3	M	3.6	Synostosis sagittal suture	CVR	371	6.1	Right parietal	Left parietal
4	M	5.2	Synostosis sagittal suture	CVR	364	6.1	Right occipital	Left occipital
5	M	5.3	Synostosis sagittal suture	CVR	325	6.4	Left anterior parietal	Right anterior parietal
6	F	1	Synostosis left lambdoid suture	CVR	394	5.9	Right parietal	Left parietal
7	M	8.8	Synostosis left lambdoid suture and synostosis sagittal suture	Re-do-CVR	351	6	Right posterior parietal and right anterior parietal	Two left anterior parietal
8	M	2.3	Synostosis sagittal suture	CVR	358	6.1	Two right occipital	Two left occipital
9	M	2	Deformational posterior plagiocephaly	CVR	363	6.4	Right parietal	Left parietal
10	F	18	Apert syndrome	Focal DBM cover	364	6	Right posterior parietal	Right anterior parietal
11	M	19	Synostosis left coronal suture	Focal DBM cover	397	5.1	Left anterior parietal and right anterior parietal	Left inferior parietal and left posterior parietal
12	M	16	Crouzon syndrome	Re-do-CVR	357	5.4	Right parietal	Left parietal
13	M	1.3	Synostosis sagittal suture	CVR	369	5.3	Left posterior parietal	Right posterior parietal
14	M	0.8	Apert syndrome	CVR	721	4.4	Central parietal posterior	Central parietal posterior
15	M	3.8	Crouzon syndrome	CVR	364	4.7	Central anterior parietal and left parietal	Central posterior parietal and right occipital
16	M	5.1	Synostosis sagittal suture	CVR	372	5.7	Left parietal and left temporal	Right parietal and right posterior parietal
17	M	3.1	Crouzon syndrome, pansynostosis	CVR	377	4.3	Left parietal and Left occipital	Left parietal and right occipital
18	F	10.4	Synostosis sagittal suture	Re-do-CVR	369	6	Left parietal and right parietal	Left anterior parietal and right posterior parietal
	Average	6.9			385	5.6		
	SD	5.8			83	0.6		

Table 2 Detailed data of 18 patients.

Patient number	Defect size at 1 week postoperatively, cm^2		Defect size at 1 year postoperatively, cm^2		Fusion degree, %		Bone dust deposition location
	DBM	Control	DBM	Control	DBM	Control	
1	20.97	15.41	4.03	7.44	80.77%	51.74%	
2	1.12	1.71	0.00	0.00	100.00%	100.00%	DBM- and control defect
	5.41	3.52	0.00	0.00	100.00%	100.00%	DBM- and control defect
3	12.86	7.06	0.72	1.92	94.41%	72.81%	Control defect
4	10.06	8.07	0.66	2.66	93.48%	67.07%	Control defect
5	23.64	15.95	2.67	11.18	88.72%	29.87%	
6	18.09	11.10	0.00	0.00	100.00%	100.00%	
7	8.55	7.30	1.44	2.81	83.13%	61.51%	
	2.79	4.26	0.21	0.21	92.52%	95.18%	
8	7.88	8.88	0.00	0.00	100.00%	100.00%	DBM defect
	7.15	3.60	0.00	0.00	100.00%	100.00%	DBM defect
9	17.28	14.04	0.00	4.59	100.00%	67.29%	Control defect
10	6.05	2.14	4.97	1.78	17.88%	16.52%	
11	6.66	8.88	5.92	8.76	11.12%	1.43%	
	6.28	7.01	7.21	6.89	−14.90%	1.72%	
12	27.90	7.30	3.67	7.31	86.84%	−0.22%	DBM defect
13	21.11	16.93	1.38	2.22	93.47%	86.88%	
14	17.92	13.25	0.66	0.81	96.32%	90.83%	
15	10.89	6.05	0.00	0.48	100.00%	91.99%	
	6.52	4.17	0.00	0.05	100.00%	98.82%	
16	5.73	6.81	3.71	6.14	35.21%	9.93%	DBM defect
	4.20	1.15	1.94	1.78	53.72%	−54.88%	DBM defect
17	11.66	5.84	2.66	3.37	77.17%	42.40%	DBM defect
	9.98	5.91	4.33	3.28	56.62%	44.54%	DBM defect
18	7.32	5.89	7.02	5.37	4.05%	8.92%	
	5.08	7.03	1.28	5.27	74.80%	25.12%	

detached pericranium was distributed equally to DBM and control sites.

Calvarial defects were covered in the secondary focal defect coverage operations by the DBM plate. In these cases, no CVR was performed and a calvarial defect was merely covered. The DBM plate was fixed with resorbable sutures to the bony borders and in direct contact with the dura.

Follow-up

3D-CT imaging was performed according to our standard protocol: 1 week and 1 year postoperatively. The patients were clinically followed up at 1 month and 1 year after surgery, with subsequent visits at the age of 3, 5, 8, and 10 years.

The mean radiological follow-up time was 385.3 days (SD 82.8) and the mean clinical follow-up time was 5.6 years (SD 0.6, range 4.3–6.4) (Table 1).

Defect area measurement

The defect areas were measured from the 3D-CT reconstructions with a developed method utilizing free open source OsiriX version 5.8.5 (OsiriX, Geneva, Switzerland). The segmentation and 3D reconstruction of the computed tomography scan DICOM (DICOM, Rosslyn, VA. USA) was performed using onboard tools of OsiriX. The threshold limit for segmentation was chosen from 200 to 3000 Houndsfield Unit (HU). Segmentation was performed with a slice of thickness 1.25 mm and isosurfaces were generated. The defect area with bony margins was separated from the skull by the built-in *scissor* tool. The defect area was opened in the 3D MPR mode (Figure 1) and slice thickness was increased to the maximum, to reveal the total area delineated by all original slices. The bone segment was manually set at an angle of 90° with respect to the anterior–posterior axle and right–left axle (Figure 1). The defect area was then positioned at 90° toward the third plane and the observer (Figure 1). This setting enabled delineation and measurement of the defect area with the built-in *pencil* tool (Figure 1).

Each defect area (with DBM and control) was independently measured from 1-week and 1-year postoperative CT images by M.S and J.T. All measurements were made in the same room using the same computer (screen: 15 inch, 4:3 aspect ratio, and XGA resolution). The average of the two measurements was used in analyses.

Calculation of the fusion degree and data analysis

The fusion degree (dF) of a defect was defined as the percentage of change of the defect area from 1 week to 12 months postoperatively. The dF values of the DBM defects
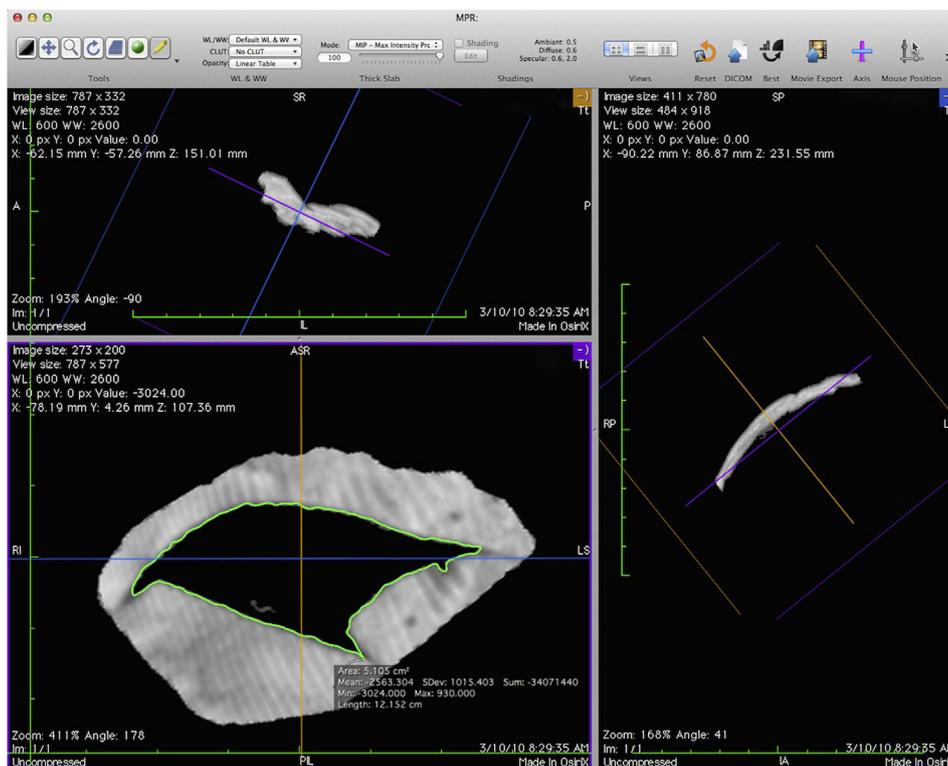
Figure 1 View in the Osirix MPR mode. Top left: a bone segment positioned at an angle of 90° with respect to the ante-rior—posterior axle. Right: a bone segment positioned at 90° with respect to the left—right axle. Bottom left: a defect area positioned at 90° toward the third plane and the observer and the defect area measurement performed by the pencil tool.

were compared with the control defects of all patients. Furthermore, the dF of the DBM defects were compared with the control defects of the patients who did not receive bone dust deposition.

An additional comparison was made separately for pa-tient groups older and younger than 30 months of age to assess the potential benefit for patients who have matured over the osteogenic potential of the dura. The 30 months of age limit was estimated by clinical experience. The corre-lation between the initial defect area and the dF was calculated. Statistical analysis of the effect of bone dust deposition on the dF was conducted. In these two analyses, DBM defects and control defects were separately compared.

Radiological observation

Bone quality-related observations were also recorded. On the basis of the results of the radiological observation, we decided to study the effect of early mineralization (EM, cloud-like white matter in the 1-week 3D CT scan) on the dF. DBM defects and control defects were separately compared.

Statistical methods

The two-tailed paired-sample Student's t-test was used to define statistical significance in the comparison between the areas and dF of the DBM and control defects (including age groups) and DBM and control defects of the patients that did not receive bone dust deposition. The Pearson correlation coefficient (r) was used to report the correlation between the

defect areas and the dF. Statistical significance was observed using the *critical value table* for r. The one-tailed two-sample Student's t-test was used to define statistical significance in the comparison between the early-mineralization groups and no-early-mineralization groups as well as the dF of bone-dust and no-bone-dust groups. For all statistical tests, statistical significance was set at $p = 0.05$.

Agreement of the two measurers (inter-rater agree-ment) and variability of the measurements of single measurer (intra-observer variability) were evaluated. Inter-rater agreement was calculated as intra-class correlation coefficient (two-way mixed absolute agreement, average measures, or ICC(3,2)) using all data points of both mea-surers. For intra-observer variability, M.S. and T.J. con-ducted repeated measurements on nine defects selected randomly (by A.R.) at least 7 days after the primary mea-surement. Intra-class correlation coefficient (two-way random absolute agreement, single measures, or ICC(2,1)) was calculated from the repeated measurements.

All statistical analyses were performed using SPSS soft-ware (v. 23, IBM Inc., Armonk, NY, USA).

Results

Defect area measurements

The mean initial areas of the DBM and control defects were 11.1 cm^2 (SD 7.1) and 7.8 cm^2 (SD 4.5), respectively (Figure 2) ($p < 0.001$).
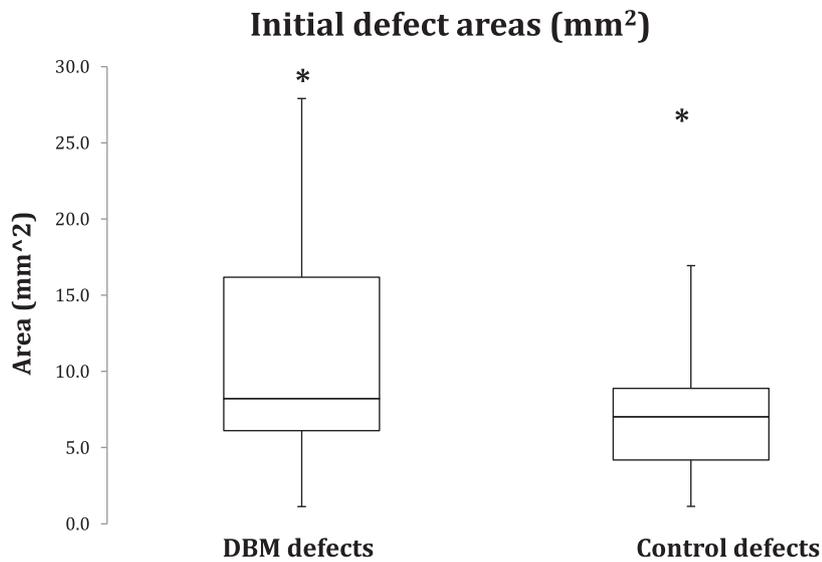
Initial defect areas (mm^2)

Figure 2 Initial mean area of the DBM and control defects.

The mean eventual areas of the DBM and control defects were 2.0 cm^2 (SD 2.29) and 3.3 cm^2 (SD 3.1), respectively ($p < 0.02$).

The dF of the defects

The mean dFs of the DBM and control defects were 74% (SD 30) and 54%, respectively (SD 43) (Table 2 and Figure 3) ($p < 0.002$).

The mean dFs of the DBM and control defects of the patients who received no bone dust deposition were 66% (SD 41.8%) and 55% (SD 39.6), respectively ($p < 0.059$).

The mean dFs of patients older than 2.3 years of DBM and control defects were 67% and 43%, respectively ($p < 0.005$).

The mean dFs of patients younger than 2.3 years of DBM and control defects were 98% and 91%, respectively ($p > 0.09$).

No correlation between the initial defect area and dF was recorded for DBM or control defects (Figures 4 and 5), r = 0.31 ($p > 0.05$) and r = 0.22 ($p > 0.05$), respectively.

Low dFs in DBM and control defects were recorded for patients 10 and 11 (Table 2). In these two patients, secondary focal defect coverage surgeries were performed.

The effect of bone dust deposition on the dF

The dFs of the bone-dust and no-bone-dust DBM defects were 79% and 72%, respectively ($p > 0.3$). The dFs of the bone-dust and no-bone-dust control defects were 81% and 48%, respectively ($p = -0.059$).

Radiological assessment

The following observations were made from 3D CTs: eight free-floating bone islands (F) in 12-month 3D CTs and 22 EM
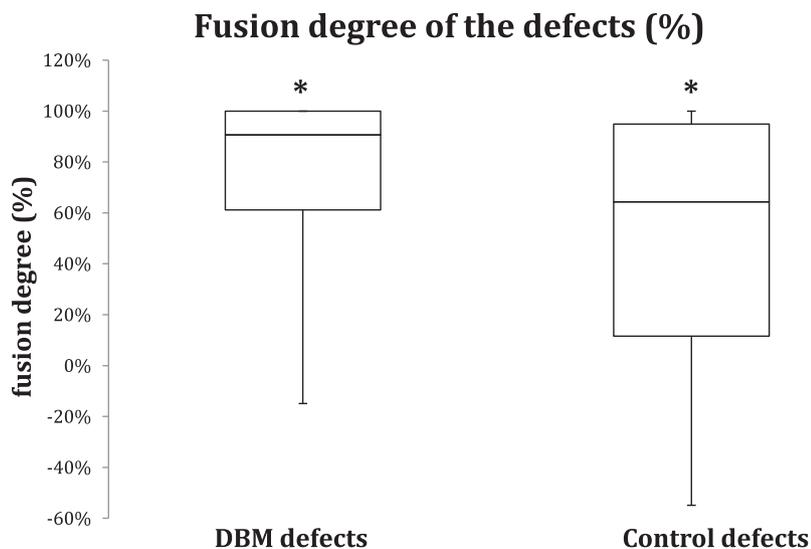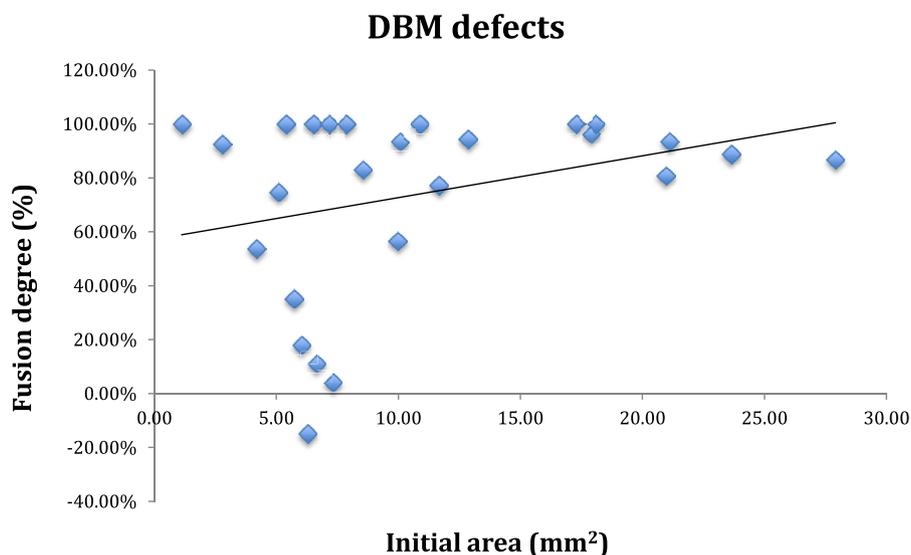
Fusion degree of the defects (%)

Figure 3 Fusion degree of the DBM and control defects.

DBM defects

Figure 4   Correlation between the DBM defect area and fusion degree.
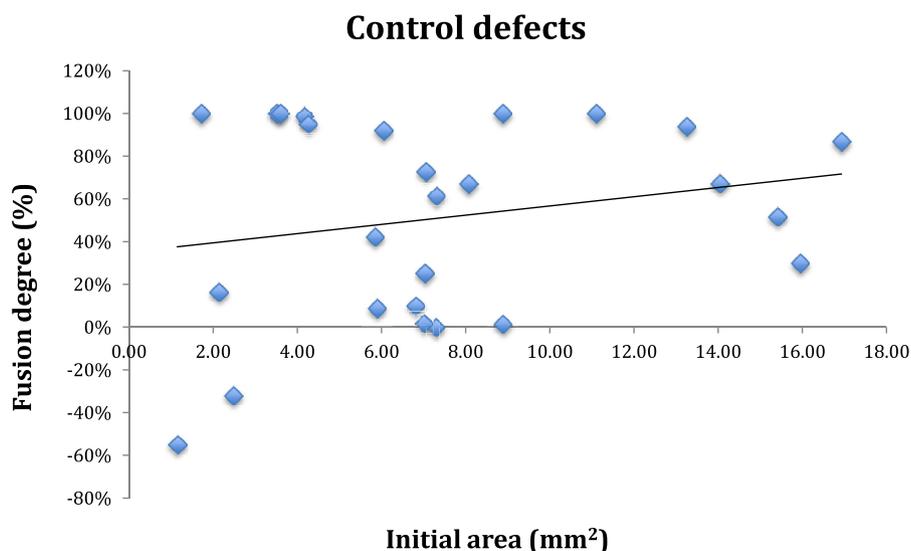
Control defects

Figure 5   Correlation between the control area and fusion degree.

(cloud-like white matter, Figure 6) in 1-week 3D CTs. Seven of these 22 cases were recorded separately from defect margins (MA) in 1-week 3D CTs (Figure 7).

The dFs of the EM and no-EM DBM defects were 86% and 54.2%, respectively ($p < 0.01$), and those of the EM and no-EM control defects were 87% and 45%, respectively ($p < 0.02$).

Complications

Signs of increased intra-cerebral pressure were not observed on any patient. No complication was recognized from the medical records and no palpable or visible abnormality at the DBM site was recorded in clinical controls. According to the medical records, DBM had not caused growth restriction on any patient. The growth trajectories did not differ from those of other CVR patients.

Reliability of measurements

The inter-rater agreement (ICC(3,2)) and intra-rater variability (ICC(2,2)) were calculated as 0.996 (0.994–0.997; 95% confidence interval) and 1.000 (0.999–1.000; 95% confidence interval), respectively.

Discussion

In this study, we compared ossification of calvarial defects covered by DBM plate with uncovered control defects identified in similar locations of the same patients' craniums. We found that the dFs of the DBM defects are higher than those of the control defects. The difference was statistically significant for patients older than 30 months. In addition, DBM appears to be more effective when it is used with bone dust. No DBM-related complication was
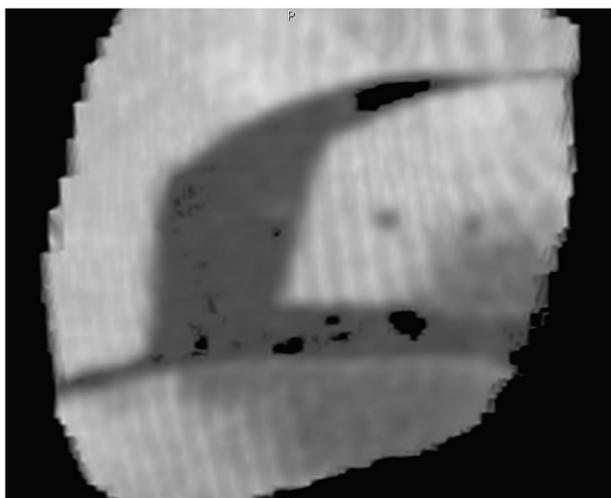
Figure 6 Early mineralization (cloud-like white matter). 1-week postoperative image, control defect patient number 2.

recorded, and therefore, it appeared to be a safe material to promote ossification.

However, the DBM plate appeared to be unable to promote extensive ossification in the majority of patients where poor ossification was also found in the control defects (Table 2). Therefore, the patients' individual osteogenic potential appears to be a significant factor determining defect fusion, and the role of DBM is more likely to support this process rather than causing bone formation. With such assumption, further studies are needed to elaborate the different osteogenic factors.

We recorded seven EM areas separate from the defect margins and eight free-floating bone islands (Figure 7). No correlation was recorded between the initial defect area and dF. In an earlier study, remineralization of DBM occurred from the periphery to the center in the adult rat critical-sized calvarial-defect model.[7] Our results suggest that the osteogenic potential of the dura induces ossification simultaneously throughout the defect area. Furthermore, our results indicate that in cases where an
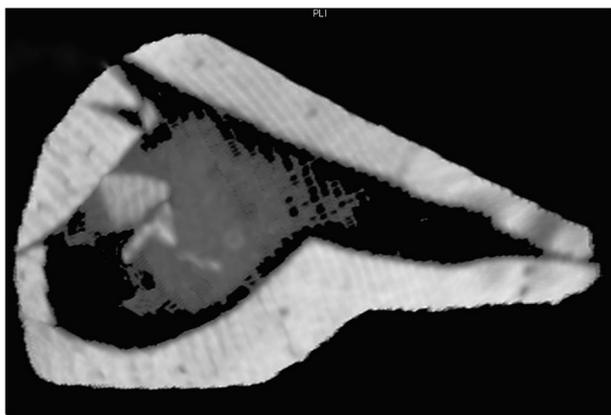

Figure 7 Independent mineralization area separate from the bone margins. A 1-week postoperative image of DBM defect of patient number 3.

osteogenic potential exists, the size of the defect area is not the dominant factor for bony fusion.

Plum et al. reported resorption of DBM putty and resorbable mesh tucked under the edges of calvarial defects in the secondary focal defect coverage surgeries.[17] Their study group consisted of five syndromic fibroblast growth factor receptor (FGFR) patients. The FGFR mutations were speculated to be a predisposing factor for poor ossification. However, in our series, the five FGFR patients did not present poor ossification. Four of them showed high dF (Table 2). On the contrary, we found resorption of DBM after secondary focal defect coverage operations, one of the two cases being associated with FGFR (Table 2). Therefore, the role of FGFR mutations in calvarial ossification remains unclear.

In focal defect coverage surgeries, DBM is fixed directly in contact with the dura. It has been shown that DBM is not suitable for use on load-bearing areas.[12,19] In light of our results, it could be hypothesized that resorption of the DBM in such cases is promoted by other factors, such as the pulsatile load of the dura, rather than merely FGFR. Furthermore, Chao et al. reported promising results using DBM putty with resorbable mesh in CVR operations, where no contact between the dura and DBM was observed.[16]

In secondary focal defect operations, the iatrogenic bony injury is not as extensive as in CVR. Injured bone promotes an ossification cascade through increased angiogenesis and growth factor release.[20—22] A recent study supports the hypothesis that mechanical strain of the dura caused by expansive growth of the brain might be an important stimulus that increases osteogenic potential.[1,23] In secondary focal defect surgeries, no calvarial vault expansion is performed, thus creating more intracranial volume for the brain to expand. The fact that the patients who underwent secondary focal defect surgeries were the oldest of our study could explain their low dF. However, our four patients with advanced age (patients 12, 18, 7, and 2), on whom re-CVR was performed, showed high dF of DBM and control defects (Tables 1 and 2). They all had matured far beyond the age of osteogenic potential of the dura. In light of these results, one could speculate that CVR might be beneficial for the patients' overall calvarial osteogenic potential.

Rodriquez et al. found that frontal sinus filled with bone dust exhibited higher bone density than frontal sinus filled with DBM and bone dust.[24] We recorded higher dF of the defects filled with bone dust than those without bone dust. The extensive dF of the control defects of patients 2, 3, and 4 with advanced age could be explained by bone-dust deposition. These findings support earlier study and our clinical experience of bone dust to be an ossification promoter, with or without DBM.[24] However, our study setup and lack of sufficient number of patients with bone dust and DBM left this suggestion inconclusive.

A cloud-like white matter with higher density than brain tissue was recorded in 22 defects in the 1-week postoperative images (Figure 6). These defects showed higher dF than other defects. This finding suggests that evolving mineralization could be detected as early as 1 week postoperatively.

Ritvanen et al. used a threshold limit from 157 to 3000 HU for segmentation of pediatric calvarial bones.[25] In

comparison, Leikola et al. used threshold limit from −90 to 155 HU for segmentation of pediatric brain tissue.[26] However, data are lacking regarding the standardized threshold limit in segmentation to produce geometrically accurate rendering of pediatric calvarium that experiences the ossification process.[2,27] Mineralization continues into early adulthood, increasing the bone density and thickness.[27] In our preliminary studies, the defect size was dependent on the chosen threshold limit. We selected 200−3000 HU for the threshold limit to ensure that all soft tissue was excluded from the 3D reconstructed skulls. We rationalized that a comparative study of the DBM and control defects within the same patient would be reliable with any significant HU value derived from the literature and measurement error tolerable with respect to the absolute size of the defects.

The developed measurement method utilizing Osirix fails to consider the varying curvature of the calvarium surface as the defect area is visualized as a 2D projection for the measurement plane. The dimension of the control defects was in the same scale and selected in as equal location as possible with the corresponding DBM defects. Hence, we estimate the error caused by this to be small relative to the results. Variations in the positioning of the bone segment and using the OsiriX *pencil tool* may cause small error in the measurements, and thus, two measurers were used. The inter-rater agreement and intra-rater variability of the measurement method and the measurements was also found to be good.

Conclusions

DBM plate is a safe and useful material to promote ossification in calvarial defects in CVR. Furthermore, DBM appears to be more effective in older patients (>30 months of age) than in younger patients or when used with bone dust. DBM might not be sufficient in secondary isolated calvarial defect coverage without concurrent CVR.

Author contributions

Study design: M.S, A.R, J.H, J.L.
    Performing retrospective review: M.S.
    Development of measurement method: M.S, A.R.
    Performing measurements: M.S, J.T, A.R.
    Data processing: M.S, A.R, J.L.
    Writing the article: all authors.

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