

Bone Generation in the Reconstruction of a Critical Size Calvarial Defect in an Experimental Model

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This study was designed to investigate the optimal combination of known osteogenic biomaterials with shape conforming struts to achieve calvarial vault reconstruction, using a canine model. Eighteen adolescent beagles were divided equally into 6 groups. A critical-size defect of 6 × 2 cm traversed the sagittal suture. The biomaterials used for calvarial reconstruction were demineralized perforated bone matrix (DBM), recombinant human bone morphogenetic protein 2 (rhBMP2), and autogenous platelet-rich plasma (PRP). The struts used were cobalt chrome (metal) or resorbable plate. The groupings were as follows: 1) DBM + metal, 2) DBM + PRP + metal, 3) DBM + PRP + resorbable plate, 4) DBM + rhBMP2 + metal, 5) DBM + rhBMP2 + PRP + metal, and 6) DBM + rhBMP2 + resorbable plate. Animals were killed at 3 months after surgery. There was no mortality or major complications. Analysis was performed macroscopically and histologically and with computed tomography. There was complete bony regeneration in the rhBMP2 groups only. Non-rhBMP2 groups had minimal bony ingrowth from the defect edges and on the dural surface, a finding confirmed by computed tomographic scan and histology. Platelet-rich plasma did not enhance bone regeneration. Shape conformation was good with both metal and resorbable plate.

rhBMP2, but not PRP, accelerated calvarial regeneration in 3 months. The DBMs in the rhBMP2 groups were substituted by new trabecular bone. Shape molding was good with both metal and resorbable plate.

Key Words: Critical size calvarial defect, cranial vault reconstruction, rhBMP2, resorbable plates, metal struts

The successful separation of a pair of craniopagus twins resulted in significant cranial vault defects that required reconstruction.¹ Previously, calvarial reconstruction in separated craniopagus twins was postponed until adolescence when autologous bone was more plentiful.² The goals of pediatric calvarial reconstruction are to provide protection for the brain, to enable normal growth of the cranial vault, and to reduce deformity of the head shape. Several principles are applicable in pediatric calvarial reconstruction. Autologous bone is the material of choice. Alloplastic materials such as hydroxyapatite are less commonly used because of a lack of osseointegration and a tendency to develop foreign body reactions. Demineralized bone matrix (DBM) is widely used by the authors and has been proven to be safe when obtained from trusted sources.³ Demineralized bone matrix is osteoinductive and osteoconductive and becomes mineralized after 1 year. However, it does not provide strength for structural support or maintenance of head shape until neo-osteogenesis is complete. Alternatively, to cover large defects, implantable materials such as titanium mesh and polyurethane custom-made implants may be considered in an adult but not in the growing child, who would outgrow these rigid materials rapidly.

Calvarial tissue engineering may potentially solve these problems. The requirements include biologic compatibility, the incorporation of a strong

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Presented at the Plastic Surgery Research Council Meeting 2005; the 10th International Congress on Cleft Palate and Related Craniofacial Anomalies 2005; and the 11th Biennial International Conference of the International Society of Craniofacial Surgery 2005.

framework, as well as being able to achieve this in a timely fashion. Biologic compatibility can be achieved by using DBM. To accelerate slow DBM osteogenesis, recombinant human bone morphogenetic protein 2 (rhBMP2) and platelet-rich plasma (PRP) were selected for evaluation. Potential scaffolding materials evaluated included nonresorbable (cobalt chrome) and resorbable (resorbable plate) materials.

The canine model with a critical-size calvarial defect was used to duplicate the clinical problem of reconstructing a large bone deficit with the need for concomitant three-dimensional structural integrity. The term *critical size* will be used to describe a defect much larger than a standard critical-size defect. The aim of this project was to investigate the best possible combination of biomaterials (DBM, rhBMP2, PRP) and scaffolding materials (cobalt chrome, resorbable plate) in calvarial reconstruction of a critical-size defect in a canine model.

MATERIALS AND METHODS

Materials

Demineralized bone matrix was prepared by the Pacific Coast Tissue Bank, Los Angeles, CA.^{4,5} They were obtained from canine tibial cortices measuring about 3 × 2 cm each. The DBM was soaked in a solution of normal saline (500 mL), vancomycin (500 mg), gentamicin (40 mg), and cefazolin (1 g) for at least half an hour before use. They were then cut to achieve a good fit and placed within the defect.

The concentration of rhBMP2 used was 0.4 mg/mL, based on the previous work of Bragdon et al.⁶ The carrier material used was a type I bovine absorbable collagen sponge (Helistat, Integra Life-Sciences Corp, Plainsboro, NJ) measuring 5 × 2.5 cm, included with the kit. The excess width was used at either end to fill the deficient length, to create a piece of sponge measuring 6 × 2 cm in size. The volume of rhBMP2 infused onto the sponge was determined according to the instructions of the Infuse (Medtronic Sofamor Danek, Memphis, TN) kit. Each sponge was infused with 1.4 mL of the solution, which was equivalent to 0.56 mg of rhBMP2. The sponge was soaked with rhBMP2 for at least 15 minutes before use.

Platelet-rich plasma was prepared using 27 mL of autogenous blood mixed with 3 mL of acid citrate dextrose solution A. This was then infused into the gravitational platelet separation system (GPS System, Biomet Biologies Inc., Warsaw, IN) and spun at 3200 rpm for 12 minutes, resulting in the separation of 3 mL of PRP, which was aspirated into a syringe. A separate syringe was prepared containing 5 mL of calcium chloride 10% with 5000 U of thrombin.

Using the GPS spray kit, both syringes were assembled and, when simultaneously depressed, squirted out the PRP, which coagulated when it came in contact with the thrombin-calcium chloride solution. The GPS system has been proven to concentrate and release a variety of potentially therapeutic growth factors.⁷

The cobalt-chrome struts measured 7.6 cm × 3.5 mm × 0.5 mm and 3 cm × 3.5 mm × 0.5 mm. Perforations were made at regular intervals. Cobalt chrome was selected because of its strength and reduced rate of bony integration.^{8,9} This was important because it may be necessary to perform a second surgery for explantation.

The resorbable plate material, Lactosorb (Biomet Sports Medicine, Inc., Warsaw, IN), was selected based on its well-proven use as a resorbable material used for bone fixation.¹⁰ Each defect would be repaired using 2 resorbable plate struts each measuring 3 × 2 × 0.2 cm. The triangular spaces in the struts were filled with pieces of DBM (as illustrated in Table 1).

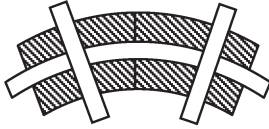
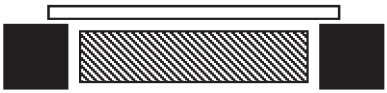
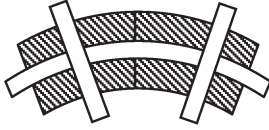
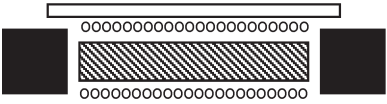

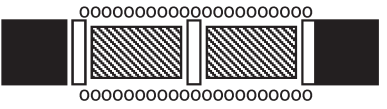
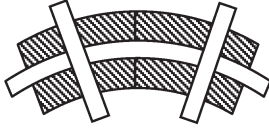
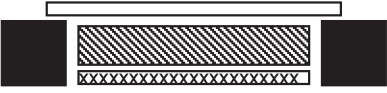
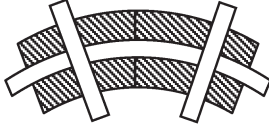
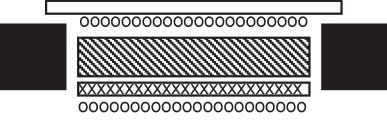

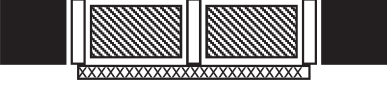
All procedures comply with the ethical guidelines for use of animals set out by the National Institutes of Health. Institutional approval was obtained for the use of eighteen 9-month-old medical-grade 2 male beagles. Critical-size calvarial vault defects of 6 × 2 cm traversing the midline were created. The mean age of the dogs was 9.17 months (range, 9–10 months) at surgery. They weighed an average of 13.4 kg (range, 12.2–14.9 kg) at the time of necropsy.

Methods

General anesthesia was administered during surgery. Preanesthesia medications given were subcutaneous or intramuscular 0.01 mg/kg glycopyrrolate, 0.2 mg/kg midazolam, 0.2 mg/kg hydromorphone, and 6 mg/kg gentamicin. Induction of anesthesia was obtained with sevoflurane and oxygen via mask. Each dog was intubated and maintained on isoflurane 1.5% to 3.5% and oxygen. Perioperatively, intravenous methylprednisolone 30 mg/kg was given as the calvarial defects were made. Postoperatively, intravenous methylprednisolone 15 mg/kg, hydromorphone 0.2 mg/kg 4 to 6 hourly then as needed, midazolam 0.1 to 0.3 mg/kg per hour for 2 hours, and subcutaneous gentamicin 6 mg/kg was given for 5 days. The dogs were randomly divided into 6 groups with 3 dogs each. The first 3 groups were the non-rhBMP2 groups, whereas the next 3 groups were the rhBMP2 groups (Table 1).

In group 1, the defect was reconstructed with 2 pieces of DBM and overlaid with cobalt-chrome struts.

Table 1. Description of Experimental Groups

Group	Superior view	Cross-section view
1. PDBM + metal		
2. PDBM + metal + PRP		
3. PDBM + Lactosorb + PRP		
4. PDBM + metal + BMP		
5. PDBM + metal + BMP + PRP		
6. PDBM + Lactosorb + BMP		

oooooooo = PRP; xxxxxxxx = BMP

A total of 3 cobalt-chrome struts were used; 1 measuring 7.6 cm × 3.5 mm × 0.5 mm would span the defect in the coronal plane; another 2 struts measuring

3 cm × 3.5 mm × 0.5 mm spanned either side of the defect in the parasagittal plane. These struts were fixed to the defect edges with 1.0-mm screws (Fig 1).

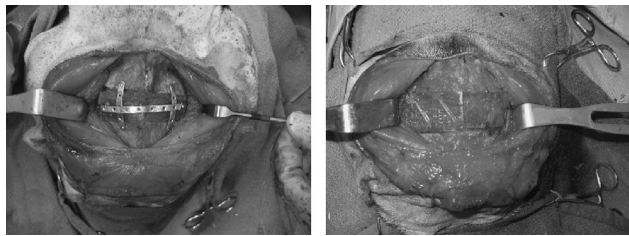


Fig 1 Left, Group 1 calvaria showing the location of the defect, insertion of 2 pieces of DBM, and placement of metal struts. Right, Group 3 calvaria showing the resorbable plate framework with inlay pieces of DBM.

In group 2, the defect was first coated with PRP on the dural surface, then 2 pieces of DBM were laid above it followed by the cobalt-chrome struts and another layer of PRP. Fixation of the struts was similar to group 1.

In group 3, the resorbable plates were soaked in normal saline of 65°C and then bent to the contour of the defect. The DBM was then cut into triangles to fit into the framework. Each piece of DBM was individually anchored to the struts using Monocryl 3-0 sutures. The dural surface of the defect was first coated with PRP and then repaired with the resorbable plate/DBM implant. Implants were fixed to the defect edges using Prolene 6-0 sutures. A final layer of PRP was then applied over the implant (Fig 1).

In group 4, the defect was repaired with rhBMP2 and DBM and overlaid with cobalt-chrome struts. The collagen sponge soaked with rhBMP2 was placed on the dura, followed by DBM. Fixation of the cobalt-chrome struts was similar to group 1.

In group 5, the dural surface of the defect was first coated with PRP followed by rhBMP2-soaked collagen sponge. The DBM was then placed over the sponge, overlaid with cobalt-chrome struts and another layer of PRP. Fixation of the cobalt-chrome struts was similar to group 1.

In group 6, the defect was repaired with a rhBMP2-soaked collagen sponge placed on the dura, followed by the resorbable plate/DBM implant. Fixation of the resorbable plate was similar to group 3.

All animals were killed at 3 months after surgery with an overdose of intravenous sodium pentobarbital (4 mL of 390 mg/mL).

Analysis

The specimens were evaluated macroscopically; by computed tomography (CT) scans and histologically. The CT scans were obtained with a GE Medical

Systems LightSpeed 16 scanner, at 120 kV, 100 mA, with a slice thickness of 0.625 mm and pixel size of 0.215 mm. The images were then reconstructed in three-dimensional volume rendering images using the Vworks 4.0 program (Cybermed, Inc, Seoul, Korea), and the amount of bone regeneration was calculated based on segmentation above 226 Hounsfield units (HU).^{11,12} To avoid scatter induced by the metal struts, they were removed before CT analysis. Statistical analysis was performed using SPSS v11.5.0 with 1-way analysis of variance and Tukey post hoc multiple comparisons.

For histologic examination, cobalt chrome and resorbable plates were removed from the excised calvarias, and segments of the specimen were prepared for undecalcified examination. Tissues were dehydrated and embedded in methylmethacrylate before sectioning on a circular saw and grinding to the appropriate thickness. The undecalcified sections

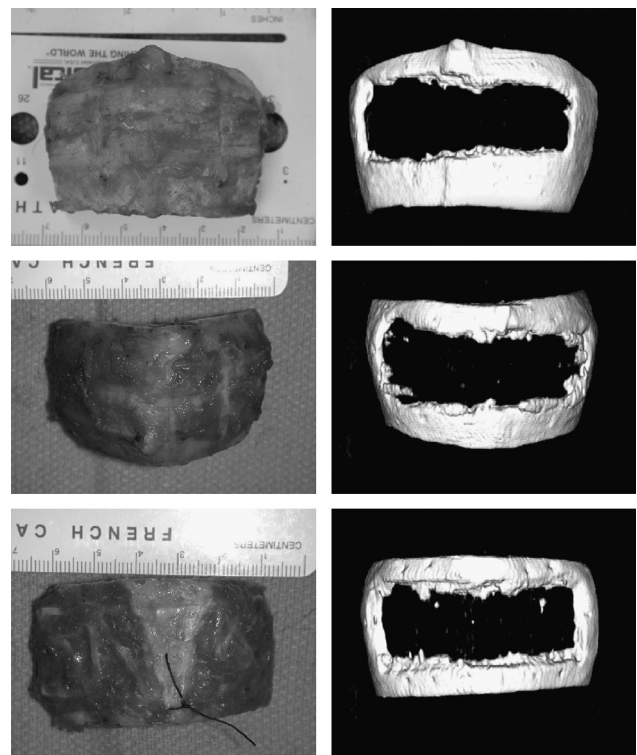


Fig 2 Non-rhBMP2 groups showing explanted specimens on the left, and CT scan thresholding for bone on the right. Group 1 (DBM and cobalt chrome) is shown in the top 2 panels. Group 2 (PRP, DBM, and cobalt chrome) is shown in the middle panel, and Group 3 (DBM and resorbable struts) is shown in the bottom 2 panels. The top of the figure is toward the face, and the bottom toward the spine. Note the absence of CT detectable bone in all groups.

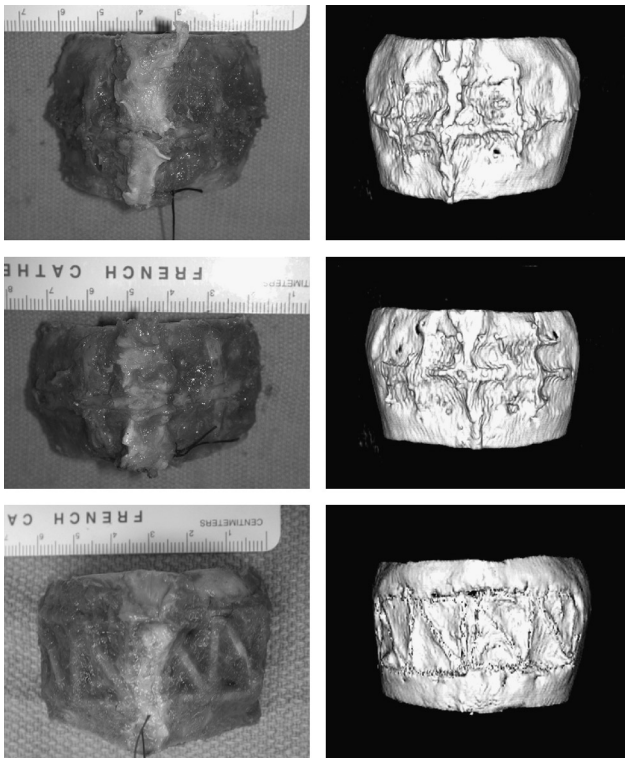


Fig 3 rhBMP2 groups showing healed calvarial defects on the left, and CT scan thresholding for bone on the right. Group 4 (rhBMP2, DBM, and cobalt chrome) is shown in the top 2 panels. Group 5 (rhBMP2, PRP, DBM, and cobalt chrome) is shown in the middle panel, and Group 6 (rhBMP2, DBM, and resorbable struts) is shown in the bottom 2 panels. The figures are oriented with the top of the figure toward the face and the bottom toward the spine. Group 4 and 5 specimens show concavities in the regions exposed to muscle, and a midline ridge. Group 6 specimen shows similar concavities and imprint of overlying the resorbable plate framework. Note extensive presence of bone in the presence of rhBMP2 in all groups.

were stained with Stevenell’s blue with micro-fuchsiun counterstain. The analysis focused on the microscopic examination of the interaction of new bone with the framework materials and the presence or absence of new trabecular bone within the defect and its boundaries.

RESULTS

All animals recovered well without suffering any major complications. One dog had a seroma under the scalp flap that manifested after 1 week and resorbed after 2 weeks. No visible suture was detected on gross examination in any of the animals, and this was confirmed in the histologic examinations.

Gross Appearance of Non-rhBMP2 Groups

In groups 1 (DBM + cobalt chrome) and 2 (DBM, PRP, and cobalt chrome), the pieces of DBM integrated well in the midline and with the edges of the defect (Fig 2). However, the central areas were not ossified. In group 3 (DBM and resorbable plate), there was fibrous tissue visible to the naked eye between the resorbable plate, the DBM, and the defect edges, and these areas were mobile.

Gross Appearance of rhBMP2 Groups

Groups 4 (rhBMP2, DBM + cobalt chrome) and 5 (rhBMP2, DBM, PRP + cobalt chrome) were grossly similar, and the entire defect had complete bone regeneration (Fig 3). The defect area was hard and was resistant to deformation. However, the bone regenerate was uneven; immediately beneath the cobalt-chrome struts were thick ridges of bone, whereas bone regenerate that was exposed to the overlying temporalis muscles had depressions. In addition, in the midline, a thick ridge of bone had overgrown the cobalt-chrome strut. This resembled the original sagittal ridge that was removed. The underlying brain appeared normal with no ectopic calcifications. Group 6 had complete calvarial regeneration as well. The superior surface of the bone regenerate showed depressions corresponding to exposure to the overlying temporalis muscle. Resorbable plates were not incorporated within the neocalvaria. All calvarial specimens had good contours

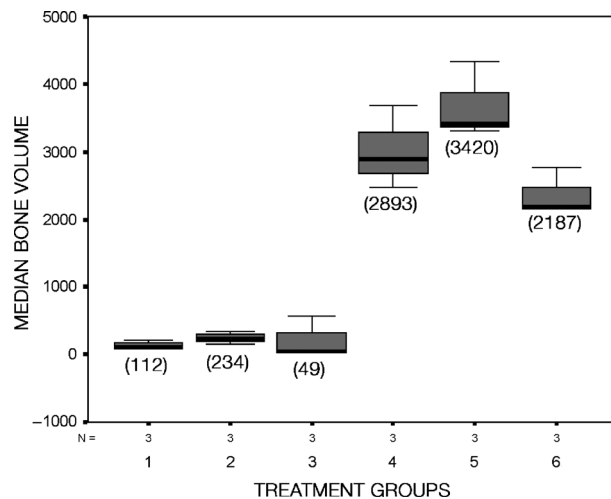


Fig 4 Graph of a box plot of the median and interquartile ranges, revealing a great disparity in bone formation (in cubic millimeters) between calvarial regeneration of the non-rhBMP2 versus the rhBMP2 groups.

Table 2. One-Way Analysis of Variance With Tukey Post Hoc Multiple Comparisons With the Dependent Variable Being the Bone Volume

Group A	Group B	Mean Difference (A-B)	SE	P	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-101.0647	319.28310	0.999	-1173.5114	971.3820
	3	-80.2307	319.28310	1.000	-1152.6774	992.2160
	4	-2875.9847*	319.28310	0.000	-3948.4314	-1803.5380
	5	-3548.4503*	319.28310	0.000	-4620.8970	-2476.0036
	6	-2240.6533*	319.28310	0.000	-3313.1000	-1168.2066
2	1	101.0647	319.28310	0.999	-971.3820	1173.5114
	3	20.8340	319.28310	1.000	-1051.6127	1093.2807
	4	-2774.9200*	319.28310	0.000	-3847.3667	-1702.4733
	5	-3447.3857*	319.28310	0.000	-4519.8324	-2374.9390
	6	-2139.5887*	319.28310	0.000	-3212.0354	-1067.1420
3	1	80.2307	319.28310	1.000	-992.2160	1152.6774
	2	-20.8340	319.28310	1.000	-1093.2807	1051.6127
	4	-2795.7540*	319.28310	0.000	-3868.2007	-1723.3073
	5	-3468.2197*	319.28310	0.000	-4540.6664	-2395.7730
	6	-2160.4227*	319.28310	0.000	-3232.8694	-1087.9760
4	1	2875.9847*	319.28310	0.000	1803.5380	3948.4314
	2	2774.9200*	319.28310	0.000	1702.4733	3847.3667
	3	2795.7540*	319.28310	0.000	1723.3073	3868.2007
	5	-672.4657	319.28310	0.345	-1744.9124	399.9810
	6	635.3313	319.28310	0.400	-437.1154	1707.7780
5	1	3548.4503*	319.28310	0.000	2476.0036	4620.8970
	2	3447.3857*	319.28310	0.000	2374.9390	4519.8324
	3	3468.2197*	319.28310	0.000	2395.7730	4540.6664
	4	672.4657	319.28310	0.345	-399.9810	1744.9124
	6	1307.7970*	319.28310	0.014	235.3503	2380.2437
6	1	2240.6533*	319.28310	0.000	1168.2066	3313.1000
	2	2139.5887*	319.28310	0.000	1067.1420	3212.0354
	3	2160.4227*	319.28310	0.000	1087.9760	3232.8694
	4	-635.3313	319.28310	0.400	-1707.7780	437.1154
	5	-1307.7970*	319.28310	0.014	-2380.2437	-235.3503

*The mean difference is significant at the 0.05 level.

conforming to the normal convexity of the beagle skull. All specimens had adherent dura on the undersurface with ridging due to normal brain contours.

CT Scans

The CT scans confirmed that only the rhBMP2 groups had complete calvarial regeneration (Fig 3). In the non-rhBMP2 groups, it was found that there were islands of bone formation at the dural surface, as well as bony ingrowth from the calvarial edges (Fig 2). Accurate determination of the volume of bone regenerate produced by the implants was made with a threshold placed above 226 HU. A box plot of the median and interquartile ranges revealed the disparity in calvarial regeneration between non-rhBMP2 and rhBMP2 groups (Fig 4). One-way analysis of variance and Tukey post hoc multiple comparisons revealed that there was a statistically significant increase in bone volume regeneration between non-rhBMP2 and rhBMP2 groups. Within

the rhBMP2 groups, group 5 had statistically more bone volume regeneration than group 6 (Table 2).

Histology

The histologic findings correlated well with the gross and CT examinations. Histologic findings showed little differences in amounts of demineralized bone and degree of mineralization within rhBMP2 and non-rhBMP2 groups. However, marked differences in the amount of original demineralized bone and degree of newly mineralized tissue could be seen between rhBMP2 and non-rhBMP2 groups. In the non-rhBMP2 groups, the formation of new trabecular bone was poor, and persistence of the DBM in its original demineralized form was found (Fig 5, left panel). In the rhBMP2 groups, all histologic sections showed that areas of new, mineralized trabecular bone had completely replaced the original DBM (Fig 5, right panel). The bone-defect interface was also clearly ossified (not shown). The new bone appeared as outer and inner tables of more compact bone

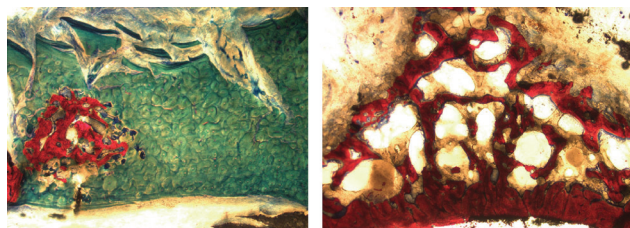


Fig 5 Photographs of histologic sections representative of non-rhBMP2 and rhBMP2 groups. Left, Representative photograph of a defect from the non-rhBMP2 group showing the edge of the defect bordering on the host bone (just visible on the extreme left of the photo). Remnants of demineralized bone (green) can be seen filling the defect, and a small island of newly formed bone (red) is visible near the edge of the defect. Right, Representative photograph showing tissue seen at the center of defects in all sections taken from the rhBMP2 groups. Dense new trabecular bone is present in the defect, with newly mineralized tissue (red) stained using alizarin red.

similar to normal calvarial bone, with an intervening area of trabecular bone of variable thickness. The outer and inner tables of bone can be seen outlining the trabecular bone (Fig 5, right panel).

The addition of PRP had no histologically detectable effect on either bone formation or vascularity of the surrounding tissues, either in the presence or absence of rhBMP2, or in the presence of cobalt-chrome or resorbable struts.

All cobalt-chrome and resorbable plating was removed before histologic analysis. There was no histologic evidence that the cobalt plates affected bone formation, although sections close to the position of the plates could not be obtained. This was due to the fact that the plates had to be removed from the bone. In the specimens with resorbable struts, there were histologically visible tissue gaps throughout the areas where resorbable plates were located. In group 6 (rhBMP2 and resorbable plate), there was a fibrous tissue envelope surrounding each strut, and bone was found beneath the resorbable plate close to the dura (not shown). The area occupied by the resorbable plates did not show any sign of resorbable plate fragmentation or any evidence of new bone growing into the area surrounding the resorbable plates.

DISCUSSION

Demineralized bone matrix has been used in the canine skull¹³ and has been reported extensively in clinical cases.^{2,3,14} Clinically, it is a good implant with an inherent content of bone morphogenetic proteins and is both osteoinductive and

osteoconductive. Demineralized bone matrix lacks antigenicity and infectivity due to the method of processing and has the ability to provide structural support and long-term osteointegration of the calvaria. However, when DBM is used alone in large and subtotal cranial defects, it may require at least 1 year before it becomes mineralized.

Bone morphogenetic proteins (BMPs) are differentiation factors belonging to the transforming growth factor b superfamily and cause mesenchymal cells to differentiate (mature) into bone- and cartilage-forming cells.^{15,16} Bone morphogenetic proteins have been proven to be efficacious in healing of calvarial defects in animal models.^{17,18} Although BMPs are composed of various subtypes, only one—rhBMP2 in the commercial form of Infuse bone graft marketed by Medtronic Sofamor Danek—is approved by the Food and Drug Administration. Infuse is osteoinductive and is used in spinal fusion.¹⁹ In this experiment, complete calvarial vault regeneration was seen only in the groups with rhBMP2. Two factors were responsible for this: osteoinduction from rhBMP2 that acted on dural pericytes, inducing them into the osteogenic lineage,²⁰ and osteoconduction via the DBM. Interestingly, the calvarial bone regenerate was not uniform. Instead, the unifying feature was that exposure of the bone regenerate to the overlying temporalis muscle led to bone resorption. There was no muscle over the midline, as this was where the temporalis muscle was split to gain access to the skull and then repaired. In the midline, there was a thick ridge of bone that grew over the metal strut and had to be removed with a rongeur before the metal strut could be explanted. When there was direct contact between the muscle and the bony regenerate, there were concavities in the bony regenerate. This could be explained by increased remodeling of the bone regenerate due to increased vascularity from the muscle. However, there was no histologic evidence to support this idea. The irregular bone formation at the metal strut areas is likely due to compression from the muscle, and it is likely that fixation can be optimized to achieve a more homogenous stress distribution. The DBM protected by overlying metal struts retained their thickness, and the metal struts did not interfere with bone formation.

Preparations such as PRP (a major source of platelet-derived growth factor) and transforming growth factor b have been reported to enhance bone grafts.²¹ These growth factors promote angiogenesis and osteogenesis, cause cells to divide, and augment production of cellular products such as extracellular matrix proteins.^{22–24} However, this effect may not be long-lasting. Wiltfang et al²⁵ found enhancement of autogenous bone healing with PRP at 2 weeks and no

later than 4 weeks. There was also no enhancement with xenogenic bone substitutes. Other studies have also not consistently shown enhancement of bone regeneration.²⁶⁻²⁹ When combined with BMPs, no synergistic or additive effect was shown. In fact, some investigations suggested that the combination of BMP and PRP might be antagonistic.^{14,15,30,31} In this experiment, PRP versus control, and PRP + rhBMP2 versus rhBMP2 were compared. The authors found that PRP did not have an added effect on bone regeneration. This finding is in concordance with that of Roldan et al,²⁸ who found that when autogenous bone was used, rhBMP7 and PRP were not better than the control group.

The second issue was to select an appropriate framework for shaping the calvarial vault. Based on his experiments, Schmitz and Hollinger³² reported that a 20-mm-diameter calvarial defect could be used to define a critical-size defect of the calvaria in dogs. To create an experimental model that would simulate the large three-dimensional calvarial vault defect of the twins, the authors designed a critical-size calvarial defect measuring 6 × 2 cm traversing the midline sagittal suture. The necessity of this design was to test the integrity of the implants over a contoured defect.

The disadvantage to the use of cobalt chrome was that it would require explantation, whereas resorbable plate would be dissolved over time. A second factor against the use of cobalt chrome was that it became incorporated into the bone regenerate in the midline. In comparison, the resorbable plate framework was always separated from the new bone by fibrous tissue.

Lactosorb resorbable plating is a 82:18 mixture of poly lactic and glycolic acids polymerized to about 100-kd polymer chains with a polydispersity of 2.2 and an inherent viscosity of 1.5 dL/g. Hydrolysis of the resorbable plate progresses after implantation, and when the inherent viscosity reaches 0.7 dL/g, from weeks 6 to 12, its strength weakens rapidly and continues, reaching zero strength by about 12 to 15 weeks. Complete resorption of the resorbable plate ranges between 9 and 15 months after surgery.¹⁰ However, there have been some biocompatibility issues with resorbable implants, ranging from foreign body reactions to reduced osteogenesis.³³⁻³⁸

The design of the resorbable plate implant was based on the geodesic dome concept of R. Buckminster Fuller, the inventor and architect who discovered that if a spherical structure was created from triangles, it would have unparalleled strength with the use of the least amount of materials. This concept was important in the reconstruction of the calvarial vault, as it was essentially a hemispherical defect.

Resorbable plate was used to form the struts of the geodesic framework, and the inner triangular pieces were composed of pieces of DBM. This achieved both a strong framework and maximal exposure of the DBM to vascular tissues such as dura and pericranium, which serve as important sources of mesenchymal stem cells.

In group 6, the bone regenerate was more compact and resembled natural cranial bone, with the presence of meningeal vessels and normal dural attachments. In comparison, when DBM was used alone in the clinical setting, there was increased adherence of the dura to the DBM, which made it difficult to separate, and may be a problem if the DBM needed to be removed. Elsalanty et al³⁹ analyzed these specimens and found that the trend for bone density and elastic modulus was higher in the presence of resorbable plate, but this difference was not statistically significant.³⁹

The authors had hoped that the resorbable plate implant would be resorbed at the same rate at which bone regeneration occurred. But, in the experiment, the bone regenerated within 3 months, leaving an essentially intact resorbable plate framework. Variable polylactic and polyglycolic acid combinations exist, and these resorbable plates can be customized to resorb at different rates. Therefore, the effects of early versus late plate resorption can be studied in the future.

A major difficulty with this study was performing statistical analysis with small sample sizes, many groups, and different variables. The statistical analysis must be interpreted with care. The great disparity between non-rhBMP2 and rhBMP2 groups strongly suggested that rhBMP2, being the common denominator, was responsible for bone regeneration. However, when comparing between groups 5 and 6, the authors were unsure as to what caused the difference in bone regeneration. This can be investigated in future studies as well. Because of the large study size, we minimized the number of groups examined. For that reason, we did not include the DBM + Lactosorb group. This was because DBM was not expected to mineralize by the end of the experiment, and the Lactosorb plate would not be resorbed as well, so we did not expect any differences between fixation with metal or Lactosorb groups. We also did not include the DBM+ rhBMP2 + PRP + Lactosorb group because we mainly wanted to see whether there was any reaction of rhBMP2 with Lactosorb.

In conclusion, the use of rhBMP2 accelerated the DBM-mediated calvarial reconstruction in 3 months. There was definite macroscopic and microscopic

differences in the amount and quality of bone regenerated. Addition of PRP had no significant positive effect on bone regeneration in this study. Shape molding was good using either cobalt-chrome or resorbable plate struts. Cobalt chrome was rigid and easily explantable, although the midline portion was incorporated within the bone regenerate. The resorbable plate was minimally resorbed at 12 weeks. The resorbable plate framework showed lack of integration with the calvarial edges and was not incorporated by bone regeneration.

REFERENCES

- Swift DM, Weprin B, Sklar F, et al. Total vertex craniopagus with crossed venous drainage: case report of successful surgical separation. *Childs Nerv Syst* 2004;20:607-617
- Salyer KE, Gendler E, Squier CA. Long-term outcome of extensive skull reconstruction using demineralized perforated bone in Siamese twins joined at the skull vertex. *Plast Reconstr Surg* 1997;99:1721-1726
- Salyer KE, Gendler E, Menendez JL, et al. Demineralized perforated bone implants in craniofacial surgery. *J Craniofac Surg* 1992;3:55-62
- Sigholm G, Gendler E, McKellop H, et al. Graft perforations favor osteoinduction. Studies of rabbit cortical grafts sterilized with ethylene oxide. *Acta Orthop Scand* 1992;63:177-182
- Gendler E. Perforated demineralized bone matrix: a new form of osteoinductive biomaterial. *J Biomed Mater Res* 1986;20:687-697
- Bragdon CR, Doherty AM, Rubash HE, et al. The efficacy of BMP-2 to induce bone ingrowth in a total hip replacement model. *Clin Orthop Relat Res* 2003;417:50-61
- Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004;114:1502-1508
- Hwang K, Schmitt JM, Hollinger JO. Interface between titanium miniplate/screw and human calvaria. *J Craniofac Surg* 2000;11:184-188
- Millar BG, Frame JW, Browne RM. A histological study of stainless steel and titanium screws in bone. *Br J Oral Maxillofac Surg* 1990;28:92-95
- Eppley BL, Morales L, Wood R, et al. Resorbable PLLA-PGA plate and screw fixation in pediatric craniofacial surgery: clinical experience in 1883 patients. *Plast Reconstr Surg* 2004;114:850-856
- Comite F, Delman M, Hutchinson-Williams K, et al. Reduced bone mass in reproductive-aged women with endometriosis. *J Clin Endocrinol Metab* 1989;69:837-842
- Lynch JA, Grigoryan M, Fierlinger A, et al. Measurement of changes in trabecular bone at fracture sites using x-ray CT and automated image registration and processing. *J Orthop Res* 2004;22:362-367
- Salyer KE, Bardach J, Squier CA, et al. Cranioplasty in the growing canine skull using demineralized perforated bone. *Plast Reconstr Surg* 1995;96:770-779
- Chen TM, Wang HJ. Cranioplasty using allogeneic perforated demineralized bone matrix with autogenous bone paste. *Ann Plast Surg* 2002;49:272-279
- Urist MR, DeLange RJ, Finerman GA. Bone cell differentiation and growth factors. *Science* 1983;220:680-686
- Wozney JM. Overview of bone morphogenetic proteins. *Spine* 2002;27:S2-S8
- Marden LJ, Hollinger JO, Chaudhari A, et al. Recombinant human bone morphogenetic protein-2 is superior to demineralized bone matrix in repairing craniotomy defects in rats. *J Biomed Mater Res* 1994;28:1127-1138
- Sheehan JP, Sheehan JM, Seeherman H, et al. The safety and utility of recombinant human bone morphogenetic protein-2 for cranial procedures in a nonhuman primate model. *J Neurosurg* 2003;98:125-130
- McKay B, Sandhu HS. Use of recombinant human bone morphogenetic protein-2 in spinal fusion applications. *Spine* 2002;27:S66-S85
- Takagi K, Urist MR. The reaction of the dura to bone morphogenetic protein (BMP) in repair of skull defects. *Ann Surg* 1982;196:100-109
- Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638-646
- Kim SG, Kim WK, Park JC, et al. A comparative study of osseointegration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. *J Oral Maxillofac Surg* 2002;60:1018-1025
- Robiony M, Polini F, Costa F, et al. Osteogenesis distraction and platelet-rich plasma for bone restoration of the severely atrophic mandible: preliminary results. *J Oral Maxillofac Surg* 2002;60:630-635
- Zhang CQ, Yuan T, Zeng BF. Experimental study on effect of platelet-rich plasma in repair of bone defect. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2003;17:355-358
- Wiltfang J, Kloss FR, Kessler P, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical-size defects. *Clin Oral Impl Res* 2004;15:187-193
- Aghaloo TL, Moy PK, Freymiller EG. Investigation of platelet-rich plasma in rabbit cranial defects: a pilot study. *J Oral Maxillofac Surg* 2002;60:1176-1181
- Aghaloo TL, Moy PK, Freymiller EG. Evaluation of platelet-rich plasma in combination with freeze-dried bone in the rabbit cranium. A pilot study. *Clin Oral Implants Res* 2005;16:250-257
- Roldan JC, Jepsen S, Miller J, et al. Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. *Bone* 2004;34:80-90
- Schlegel KA, Donath K, Rupprecht S, et al. De novo bone formation using bovine collagen and platelet-rich plasma. *Biomaterials* 2004;25:5387-5393
- Harris SE, Bonewald LF, Harris MA, et al. Effects of transforming growth factor beta on bone nodule formation and expression of bone morphogenetic protein 2, osteocalcin, osteopontin, alkaline phosphatase, and type I collagen mRNA in long-term cultures of fetal rat calvarial osteoblasts. *J Bone Miner Res* 1994;9:855-863
- Marden LJ, Fan RS, Pierce GF, et al. Platelet-derived growth factor inhibits bone regeneration induced by osteogenin, a bone morphogenetic protein, in rat craniotomy defects. *J Clin Invest* 1993;92:2897-2905
- Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orth* 1986;205:299-308
- Robinson BP, Hollinger JO, Szachowicz EH, et al. Calvarial bone repair with porous α -poly lactide. *Otolaryngol Head Neck Surg* 1995;112:707-713
- Winet H, Hollinger JO. Incorporation of polylactide-polyglycolide in a cortical defect: neoosteogenesis in a bone chamber. *J Biomed Mater Res* 1993;27:667-676
- Winet H, Hollinger JO, Stevanovic M. Incorporation of polylactide-polyglycolide in a cortical defect: neoangiogenesis and blood supply in a bone chamber. *J Orthop Res* 1995;13:679-689

36. Weber FE, Eyrich G, Gratz KW, et al. Slow and continuous application of human recombinant bone morphogenetic protein via biodegradable poly(lactide-co-glycolide) foamspheres. *Int J Oral Maxillofac Surg* 2002;31:60–65
37. Boyan BD, Lohmann CH, Somers A, et al. Potential of porous poly-D,L-lactide-co-glycolide particles as a carrier for recombinant human bone morphogenetic protein-2 during osteoinduction in vivo. *J Biomed Mater Res* 1999;46:51–59
38. Miki T, Imai Y. Osteoinductive potential of freeze-dried, biodegradable, poly (glycolic acid-co-lactic acid) disks incorporated with bone morphogenetic protein in skull defects of rats. *Int J Oral Maxillofac Surg* 1996;25:402–406
39. Elsalanty ME, Por YC, Genecov DG, et al. Histomorphometric and ultrasound analysis of bone generated from supporting and bone-enhancing materials for reconstruction of large cranial defects. *J Oral Maxillofac Surg*. In press.