

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

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### Abstract

**Objective:** 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. **Methods:** Eighteen adolescent beagles were divided equally into 6 groups. A critical size defect of 6 x 2 cm traversed the sagittal suture. The biomaterials used for calvarial reconstruction were demineralised 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 euthanised at 3 months post-surgery. There was no mortality or major complications. Analysis was performed macroscopically, histologically, and with computed tomography (CT). **Results:** 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 CT scan and histology. PRP did not enhance bone regeneration. Shape conformation was good with both metal and resorbable plate. **Conclusion:** rhBMP2 but not PRP accelerated calvarial regeneration in 3 months. The DBM in the rhBMP2 groups were substituted by new trabecular bone. Shape molding was good with both metal and resorbable plate.

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**Key words:** Critical size calvarial defect, Cranial vault reconstruction, Metal struts, Resorbable plates, rhBMP2

### Introduction

The successful separation of a pair of craniopagus twins resulted in significant cranial vault defects that required reconstruction.<sup>1</sup> Previously, calvarial reconstruction in separated craniopagus twins was postponed until adolescence when autologous bone was more plentiful.<sup>2</sup> 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. Demineralised bone matrix (DBM) is widely used by the authors and has been proven to be safe when obtained from trusted sources.<sup>3</sup> DBM is osteoinductive and osteoconductive and becomes mineralised 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 polymer custom made implants may be considered in an adult but not in the growing child, who would outgrow these rigid materials rapidly.

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Calvarial tissue engineering may potentially solve these problems. The requirements include biological compatibility, the incorporation of a strong framework, as well as being able to achieve this in a timely fashion. Biological 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 non-resorbable (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 3-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*

DBM was prepared by the Pacific Coast Tissue Bank, Los Angeles, California.<sup>4,5</sup> They were obtained from canine tibial cortices measuring about 3 x 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 prior to 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.<sup>6</sup> The carrier material used was a Type I bovine absorbable collagen sponge (Helistat®, Integra LifeSciences Corporation, Plainsboro, New Jersey), measuring 5 x 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 x 2 cm in size. The volume of rhBMP2 infused on to the sponge was determined according to the instructions of the Infuse® kit. Each sponge was infused with 1.4 mL of solution, which was equivalent to 0.56 mg of rhBMP2. The sponge was soaked with rhBMP2 for at least 15 minutes prior to use.

PRP 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) 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 units 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.<sup>7</sup>

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

The resorbable plate material Lactosorb® was selected based on its well-proven use as a resorbable material used for bone fixation.<sup>10</sup> Each defect would be repaired using 2 resorbable plate struts each measuring 3 x 2 x 0.2 cm. The triangular spaces in the struts were filled with pieces of DBM (Table 1).

All procedures comply with the ethical guidelines for use of animals set out by the NIH. Institutional approval was obtained for the use of 18 9-month-old medical grade 2 male beagles. Critical size calvarial vault defects of 6 x 2 cm traversing the midline were created. The mean age of the dogs was 9.17 months (range, 9 to 10) at surgery. They weighed an average of 13.4 kg (range, 12.2 to 14.9) at the time of necropsy.

Table 1. Description of Experimental Groups

Group	Superior view	Cross-section view
1. DBM + Metal		
2. DBM + Metal + PRP		
3. DBM + Resorbable plate + PRP		
4. DBM + Metal + rhBMP2		
5. DBM + Metal + rhBMP2 + PRP		
6. DBM + Resorbable plate + rhBMP2		
Legends	ooooooo = PRP xxxxxxx = rhBMP2	

## Methods

General anaesthesia was administered during surgery. Pre-anaesthesia 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 anaesthesia 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 PRN, midazolam 0.1 to 0.3 mg/kg/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 while 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. A total of 3 cobalt chrome struts were used; 1 measuring 7.6 cm x 3.5 mm x 0.5 mm would span the defect in the coronal plane; another 2 struts measuring 3 cm x 3.5 mm x 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).

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 30 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 60 sutures. A final layer of PRP was then applied over the implant (Fig. 1).

In group 4, the defect was repaired with rhBMP2, 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 euthanised at 3 months post-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 3-dimensional volume rendering images using the Vworks™ 4.0 program (Cybermed, Inc, Seoul, Korea), and the amount of bone regeneration calculated based on segmentation above 226 Hounsfield Units (HU).<sup>11,12</sup> To avoid scatter induced by the metal struts, they were removed prior to CT analysis. Statistical analysis was performed using SPSS v11.5.0 with one-way analysis of variance (ANOVA) and Tukey post-hoc multiple comparisons.

For histological examination, cobalt chrome and resorbable plates were removed from the excised calvariae, and segments of the specimen prepared for undecalcified examination. Tissues were dehydrated and embedded in methylmethacrylate prior to sectioning on a circular saw and grinding to the appropriate thickness. The undecalcified sections were stained with Stevenell's blue with picro-fuchsin counter-stain. 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 histological 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 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 ANOVA 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 histological findings correlated well with the gross and CT examinations. Histological findings showed little differences in amounts of demineralised bone and degree of mineralisation within rhBMP2 and non-rhBMP2 groups. However, marked differences in the amount of original demineralised bone and degree of newly mineralised 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 demineralised form was found (Fig. 5, left panel). In the rhBMP2 groups, all histological sections showed that areas of new, mineralised 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 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 prior to histological analysis. There was no histological 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

DBM has been used in the canine skull<sup>13</sup> and has been reported extensively in clinical cases.<sup>2,3,14</sup> Clinically, it is a good implant with an inherent content of BMPs, and is both osteoinductive and osteoconductive. DBM 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 mineralised.

BMPs are differentiation factors belonging to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, and cause mesenchymal cells to differentiate (mature) into bone- and cartilage-forming cells.<sup>15,16</sup> BMPs have been proven to be efficacious in healing of calvarial defects in animal models.<sup>17,18</sup> Although BMPs are composed of various subtypes, only one – rhBMP2 in the commercial form of Infuse® bone graft marketed by Medtronic Sofamor Danek, USA – is approved by the FDA. Infuse® is osteoinductive and is used in spinal fusion.<sup>19</sup> 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,<sup>20</sup> and osteoconduction via the DBM. Interestingly, the calvarial bone regenerate was not uniform. Instead, the unifying feature

Table 2. One-Way ANOVA with Tukey Post Hoc Multiple Comparisons with the Dependent Variable Being the Bone Volume

Group (A)	Group (B)	Mean difference (A-B)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
1	2	-101.0647	319.28310	.999	-1173.5114	971.3820
	3	-80.2307	319.28310	1.000	-1152.6774	992.2160
	4	-2875.9847(*)	319.28310	.000	-3948.4314	-1803.5380
	5	-3548.4503(*)	319.28310	.000	-4620.8970	-2476.0036
	6	-2240.6533(*)	319.28310	.000	-3313.1000	-1168.2066
2	1	101.0647	319.28310	.999	-971.3820	1173.5114
	3	20.8340	319.28310	1.000	-1051.6127	1093.2807
	4	-2774.9200(*)	319.28310	.000	-3847.3667	-1702.4733
	5	-3447.3857(*)	319.28310	.000	-4519.8324	-2374.9390
	6	-2139.5887(*)	319.28310	.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	.000	-3868.2007	-1723.3073
	5	-3468.2197(*)	319.28310	.000	-4540.6664	-2395.7730
	6	-2160.4227(*)	319.28310	.000	-3232.8694	-1087.9760
4	1	2875.9847(*)	319.28310	.000	1803.5380	3948.4314
	2	2774.9200(*)	319.28310	.000	1702.4733	3847.3667
	3	2795.7540(*)	319.28310	.000	1723.3073	3868.2007
	5	-672.4657	319.28310	.345	-1744.9124	399.9810
	6	635.3313	319.28310	.400	-437.1154	1707.7780
5	1	3548.4503(*)	319.28310	.000	2476.0036	4620.8970
	2	3447.3857(*)	319.28310	.000	2374.9390	4519.8324
	3	3468.2197(*)	319.28310	.000	2395.7730	4540.6664
	4	672.4657	319.28310	.345	-399.9810	1744.9124
	6	1307.7970(*)	319.28310	.014	235.3503	2380.2437
6	1	2240.6533(*)	319.28310	.000	1168.2066	3313.1000
	2	2139.5887(*)	319.28310	.000	1067.1420	3212.0354
	3	2160.4227(*)	319.28310	.000	1087.9760	3232.8694
	4	-635.3313	319.28310	.400	-1707.7780	437.1154
	5	-1307.7970(*)	319.28310	.014	-2380.2437	-235.3503

\* The mean difference is significant at the 0.05 level

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 remodelling of the

bony regenerate due to increased vascularity from the muscle. However, there was no histological evidence to support this idea. The irregular bone formation at the metal strut areas are likely due to compression from the muscle, and it is likely fixation can be optimised 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 PDGF and TGF-β) have been reported to enhance bone grafts.<sup>21</sup> These

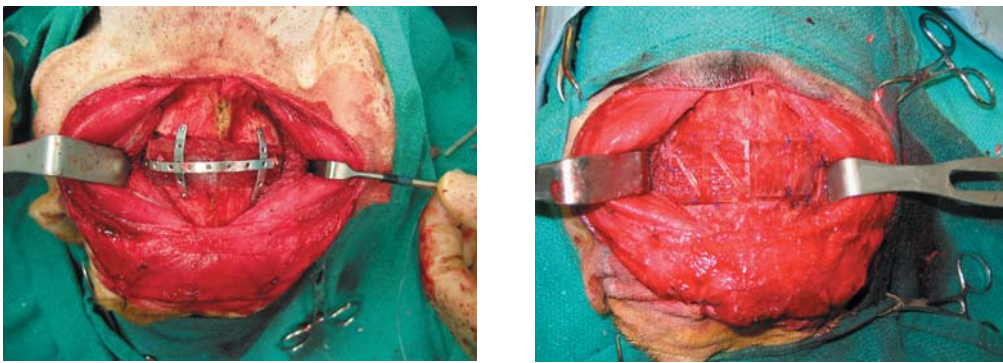


Fig 1. Left, Group 1 calvaria showing the location of the defect, insertion of 2 pieces of DBM and placement of metal struts. Right, Group 3 calvaria showing the resorbable plate framework with inlay pieces of DBM.

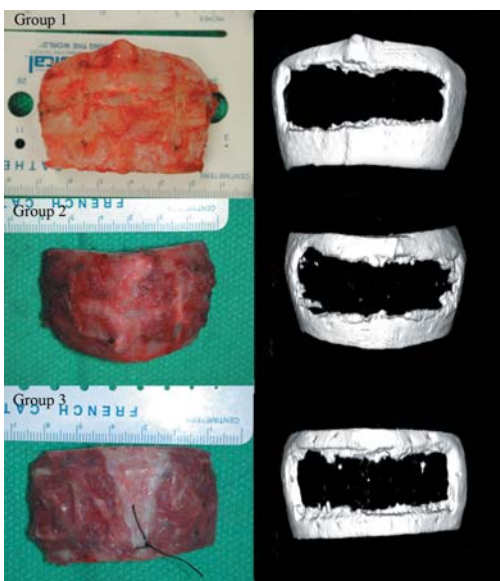


Fig 2. Non-rhBMP2 groups showing explanted specimens on the left, and CT scan thresholding for bone on the right. Group 1 (DBM and cobalt chrome) is shown in the top two panels. Group 2 (PRP, DBM and cobalt chrome) is shown in the middle panel, and Group 3 (DBM and resorbable struts) is shown in the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

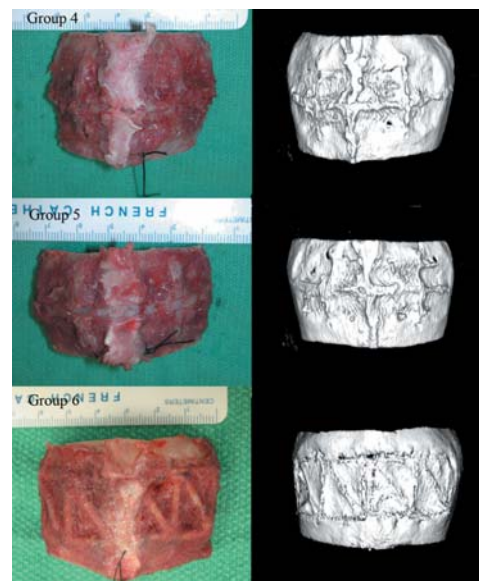


Fig 3. rhBMP2 groups showing healed calvarial defects on the left, and CT scan thresholding for bone on the right. Group 4 (rhBMP2, DBM and cobalt chrome) is shown in the top two panels. Group 5 (rhBMP2, PRP, DBM and cobalt chrome) is shown in the middle panel, and Group 6 (rhBMP2, DBM and resorbable struts) is shown in the bottom 2 panels. The figures are oriented with the top of the figure towards the face and the bottom towards 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.

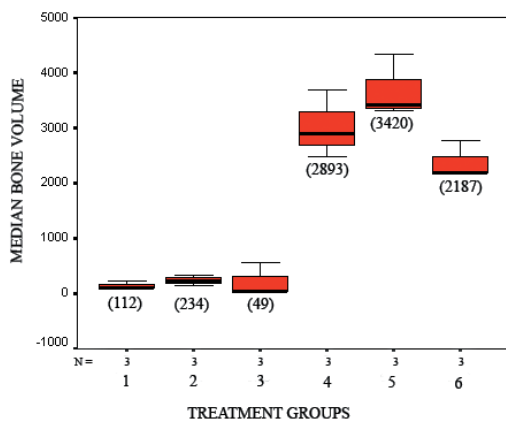


Fig 4. Graph of a box plot of the median and interquartile ranges, revealing a great disparity in bone formation (mm<sup>3</sup>) between calvarial regeneration of the non-rhBMP2 vs the rhBMP2 groups.

growth factors promote angiogenesis and osteogenesis, cause cells to divide, and augment production of cellular products such as extracellular matrix proteins.<sup>22-24</sup> However, this effect may not be long lasting. Wiltfang et al<sup>25</sup> 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.<sup>26-29</sup> 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

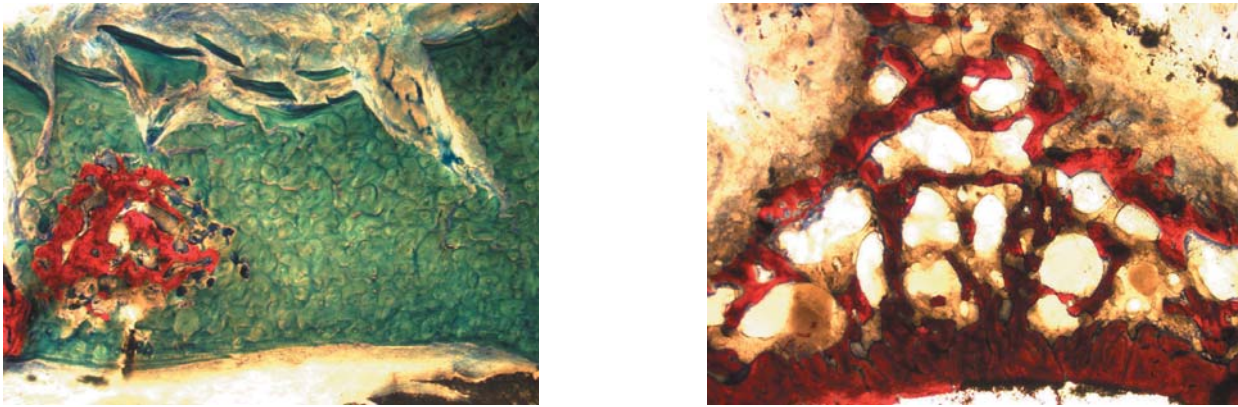


Fig 5. Photographs of histological sections representative of non-rhBMP2 and rhBMP2 groups. Left, Representative photograph of a defect from the non-rhBMP2 group showing the edge of the defect bordering on the host bone (just visible on the extreme left of the photo). Remnants of demineralised bone (green) 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 centre of defects in all sections taken from the rhBMP2 groups. Dense new trabecular bone is present in the defect, with newly mineralised tissue (red) stained using alizarin red.

antagonistic.<sup>14,15,30,31</sup> In this experiment, PRP vs control, and PRP + rhBMP2 vs rhBMP2 were compared. The authors found that PRP did not have an added effect on bone regeneration. This finding is in concordance with those of Roldan et al,<sup>28</sup> 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 their experiments, Schmitz and Hollinger<sup>32</sup> 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 3-dimensional calvarial vault defect of the twins, the authors designed a critical size calvarial defect measuring 6 x 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 polymerised to a ca. 100 kDa 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 week 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.<sup>10</sup>

However, there have been some biocompatibility issues with resorbable implants, ranging from foreign body reactions to reduced osteogenesis.<sup>33-38</sup>

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 hemi-spherical 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 al<sup>39</sup> analysed 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.

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 customised 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 minimised the number of groups examined. For that reason, we did not include the DBM + resorbable plate group. This was because DBM was not expected to mineralise by the end of the experiment and the resorbable plate would not be resorbed as well, so we did not expect any differences between fixation with metal or resorbable plate groups. We also did not include the DBM + rhBMP2 + PRP + resorbable plate group because we mainly wanted to see whether there was any reaction of rhBMP2 with the resorbable plate.

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

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