Published in final edited form as:

Plast Reconstr Surg. 2010 June ; 125(6): 1685–1692. doi:10.1097/PRS.0b013e3181cb63a3.

Testing the “critical-size” in calvarial bone defects: revisiting the concept of a critical-sized defect (CSD)

Gregory M. Cooper, PhD^{1,2,3}, Mark P. Mooney, PhD^{1,2,4}, Arun K. Gosain, MD⁵, Phil G. Campbell, PhD⁶, Joseph E. Losee, MD¹, and Johnny Huard, PhD^{3,7}

¹ Department of Surgery, Division of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA

² Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA

³ Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

⁴ Departments of Anthropology and Orthodontics, University of Pittsburgh, Pittsburgh, PA

⁵ Department of Plastic Surgery, Case Western Reserve University School of Medicine, Cleveland, OH

⁶ Institute of Complex Engineered Systems and Molecular Biosensor and Imaging Center and Departments of Biomedical Engineering, Material Science and Engineering, and Biology, Carnegie Mellon University, Pittsburgh, PA

⁷ Departments of Orthopaedic Surgery and Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA

Abstract

Background—There is a clinical need for bone replacement strategies because of the shortfalls endemic to autologous bone grafting, especially in the pediatric patient population. For the past 25 years, the animal model that has been used to test bone replacement strategies has been the calvarial critical-sized defect (CSD), based on the initial size of the bone defect. This study was undertaken to test the concept of the critical-size in several different models. A review of the theoretical and scientific bases for the CSD was also undertaken.

Methods—Two different rodent species (including 28 adult mice and 6 adult rats) were used to assess bone healing via 2D radiographic analysis after creating small bone defects using different surgical techniques.

Results—Defects in mice that were smaller than critical-sized (1.8mm diameter) were shown to heal a maximum of 50% one year postoperatively. Small (2.3mm diameter) defects in the rat skull showed approximately 35% healing after 6 weeks. Neither the choice of rodent species nor the maintenance of the dura mater significantly affected calvarial bone healing.

Conclusions—These results suggest that calvarial bone healing is not well described and much more data needs to be collected. Also, after a review of the existing literature and a critique of the clinical applicability of the model, it is suggested that the use of the term “critical-sized defect” be discontinued.

Corresponding Author: Gregory M. Cooper, Ph.D., 3510 Rangos Research Center, Children’s Hospital of Pittsburgh, 530 45th Street, Pittsburgh, PA 15201, Greg.Cooper@chp.edu.

Each of the authors listed state that there are no conflicts of interest to disclose with regards to the work presented in this manuscript.

Introduction

For the last 25 years, the large-scale calvarial defect has been used as an in vivo model to test bone replacement materials (BRMs) and bone regenerative therapies (cell-, protein-, or gene-based approaches) [1,2]. The standard rodent models are either the 8mm round defect made in the rat [3,4] or the 5mm round defect in the mouse [5]. One advantage of the calvarial defect model is that it involves healing orthotopic bone sites, making the results more physiologically relevant than those collected from bone induction in ectopic sites, such as muscle pockets or subcutaneous sites [6].

The “critical-size defect” (CSD), as these large-scale calvarial defects have become known, was originally developed as a model of craniofacial fibrous nonunion and was intended to standardize the testing of bone repair materials that could be used as alternatives to bone allo- or autografting [3]. CSDs were originally defined as “the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal” by Schmitz and Hollinger in 1986 [3]. The CSD is different from other nonunion models because it is based on the size of the defect; specifically, the CSD-dependent nonunion occurs because the calvarial defect is too large to heal with bony tissue [3]. Since the introduction of the model, CSDs have been used routinely in many laboratories to test the osteogenic capacities of different bone repair techniques (for review, see Mooney and Siegel, 2005 [7]).

One aspect of bone healing that has not been addressed is spatial control of bone formation [8]. In cases of bone overgrowth, such as craniosynostosis or fibrodysplasia ossificans progressiva, or in relation to bone regenerative therapies where the size or shape of the resulting bone is important, it is necessary to spatially control bone formation. To test different means to control bone formation, a model of normally healing bone must be developed. Such a model should reproducibly heal within a relatively short amount of time (weeks) and could be used to test different therapies designed to control bone healing within the defect.

There have been ample data collected on the healing patterns of the rodent CSD. However, there is a paucity of studies [9–11] focused on the healing of calvarial defects that are smaller than critical-size (8mm in the rat and 5mm in the mouse) [5,7,12]. Defects smaller than critical size should heal, though data showing the pattern of normal healing in the craniofacial skeleton are not readily available.

It is also well known that, among other factors, the dura mater plays a significant role in the healing of calvarial defects [13–18]. The dura mater appears to be both the primary source of osteogenic cells and the source of osteoinductive factors during calvarial wound healing [19, 20]. Surgical techniques that employ a trephine can easily damage or destroy the dura mater underlying the defect, possibly inhibiting defect healing. Therefore, healing of a calvarial defect may be influenced not only the size of the defect, but also by the manner in which it was created.

The current study sought to develop a model of normal bone healing in calvarial bone. Such a model would allow for the characterization of the cellular and molecular processes that lead to craniofacial bone healing. Bone healing was assessed in different rodent species after small bone defects were created with either a trephine or a modified, trephine and periosteal elevator-based (“elevator”), surgical technique. We tested the hypothesis, in a series of rodent studies, that defects that were smaller than the critical size would heal spontaneously in a relatively short amount of time (within weeks) especially if the dura were kept intact using the modified “elevator” surgical technique. In addition, the theoretical and scientific bases for the CSD and its use in bone tissue engineering also will be reviewed and discussed.

Materials and Methods

All animal studies were performed in accordance with federal regulations and with approval from the Animal Research and Care Committee (ARCC) at the Children's Hospital of Pittsburgh. The healing of calvarial defects was assessed over time in two different species (mouse and rat) using different surgical techniques (trephine or elevator) as described below (Table 1).

Mouse: Standard Trephine Surgery

Mouse calvarial CSDs have been traditionally defined as 5mm round defects [5,7,12]. Smaller, 1.8mm defects were created in 10, 10-week old, normal mice (C57BL-6J, Jackson Labs) using trephines (Fine Science Tools). At the time of surgery, the trephines were used to make unilateral, bicortical, mid-parietal defects. The 1.8mm trephine was chosen because it was the smallest commercially available trephine. Mice were euthanized 6 weeks after surgery to assess bone healing.

Mouse: Modified "Elevator" Surgery

We tested the effects of the surgical technique used to create the defect on healing. In the first group of animals ("trephine" group, n=18), a trephine was used to create a bicortical defect with particular attention paid to preserving the dura mater. In the second group of animals ("dural damage" group, n=18), the trephine was used to create the bone defect and the dura mater was deliberately damaged to ensure disruption of the dura. In the third group ("elevator" group, n=18), a modified surgery was performed. The 1.8mm trephine was used to score the parietal bone and cut through the ectocortex and some of the endocortex. A small periosteal otoelevator was then used to break through the remaining endocortex and to remove the bone from the defect. This technique assured the preservation of the dura mater (Figure 1A) and created a defect that was 1.8mm in diameter at the ectocortex; however, the technique also led to a defect with a variable diameter along the endocortex, because the bone was fractured instead of cut in this region (Figure 1B).

Mice in this part of the study were euthanized 4 weeks, 8 weeks, and 52 weeks (1 year) after surgery (leading to an n=6 per group per time point) and healing of the parietal defects was assessed radiographically.

Rat: Standard Trephine Surgery

The rat CSD has been reported to be 8mm [3]. Bicortical, full-thickness, defects were created using a 2.3mm outer diameter trephine in the parietal bones of 6 adult Sprague-Dawley (SD) rats and the rats were allowed to heal for 6 weeks. Six weeks after surgery, the rats were euthanized and defect healing was assessed using radiographic analysis.

Radiographic Analysis

At the end of each study, animals were euthanized, the cranial bases and brains were removed from the fixed heads, and the calvariae were radiographed using 5X magnification on a Faxitron MX-20 (Faxitron X-ray Corp, Lincolnshire, Ill.) set to 35kV and 250-second exposure on Kodak X-OMAT V film (Eastman Kodak, Rochester, NY). Developed x-ray films were scanned with a ScanMaker 9800XL (Microtek, Fontana, CA) set for radiographic scanning at 1200dpi. The scanned images were imported into Northern Eclipse software (Empix Imaging, Mississauga, Ontario, Canada) and the remaining defect area was measured. Percent healing was determined by subtracting the remaining (measured) defect area from the geometric original defect area (2.51mm^2) $\times 100$ and dividing this product by the geometric original defect area (2.51mm^2). Means were compared between groups using either t-tests (for mouse and rat

standard trephine studies) or ANOVA (for mouse modified “elevator” study) using SPSS (v12) software.

Results

Mouse: Standard Trephine Surgery

We analyzed healing of small 1.8mm diameter bone defects radiographically using 2-dimensional defect area analysis 6 weeks after surgery (Figure 2A). In the 10 animals used in this part of the study, we found large defects still remaining with an average of approximately 43.3% healing after 6 weeks (Figure 2B).

Mouse: Modified “Elevator” Surgery

Results show that the “elevator” group of mice had a greater mean bone area (smaller bone defects) at 4 weeks, 8 weeks, and 1 year postoperatively compared to the “trephine” group (Figure 3). However, there were no significant differences noted at any postoperative interval. Analysis of radiographs after 1 year of healing (Figure 3C,D) showed that calvarial defects created using the “elevator” technique were 52% filled by bone, whereas the “trephine” and “dural damage” groups healed similarly and healed no more than 35% of the defect ($p=.257$).

Rat: Standard Trephine Surgery

Radiographs taken of the 2.3mm defects in the parietal bone of Sprague-Dawley rats revealed incomplete healing after 6 weeks (Figure 4A). In fact, analysis showed that defects healed approximately 36.9% (Figure 4B).

Discussion

In order to properly test different strategies to control bone formation, a model of normal bone healing is needed. We set out to use the most widely accepted calvarial bone defect model, that of the critical-sized defect (CSD), as a basis for the development of this new model. It is well known that there are factors other than the size of the defect that influence bone healing, including the age of the patient, scarring, nutrition, etc. Because the CSD was defined on the specific size of the defect [3], with no mention of these other factors, we started to analyze the healing of different defects based on original defect size.

Defects created in the mouse that were 1.8mm diameter were found to heal approximately 30%. These data suggest that defects much smaller than critical-size do not spontaneously heal within 6 weeks. These data are supported by the observations of Cowen et al., 2004 [10] who showed in their supplementary figures that 2mm diameter defects were not healed in the mouse after 12 weeks.

In order to rule out dural damage as a cause for the lack of healing in the small defects, we developed a means to create a small skull defect in the mouse without risking damage to the dura mater. We found that the modified technique improved healing. However, this improvement did not lead to rapid, complete healing, nor was it statistically significant. Interestingly, even the best healing that was noted (in the “elevator” group) only reached approximately 50% healing. Healing seemed to plateau between 4 and 8 weeks postoperatively. This 4–8 week critical time point is supported by the findings of Gosain et al. 2000 [21] that documented a change of 51.0% healing of 3mm defects in rats after 4 weeks to 81.1% healing at 8 weeks, a difference of 30.1%. Twelve weeks after surgery, defects healed only an additional 7.7% over the 8 week time point (to 88.8% healed) [21]. Together these studies suggest that there is a critical time between the 4th and 8th week after injury in rodent models that may be sufficient to estimate the total healing that will occur, and that careful dissection of the dura

mater did not significantly influence this pattern. Therefore, we investigated the effect that animal species had on the healing of small calvarial defects.

We hypothesized that the observed lack of healing in small defects in the mouse may have been endemic to the mouse species. Therefore, we created small calvarial defects (2.3mm) in the rat model and found approximately 35% bone healing. These data suggest that we were unable to achieve the large-scale, rapid healing of a small calvarial defect by changing the species to rat. Although the rat was believed to be a robust bone-forming animal model, the data presented above suggests that small defects made in the rat parietal bone do not meet the criteria needed for a new model of craniofacial bone healing.

REVIEW

Critical Size Defects

The original “critical-size defects”—The critical-size defect (CSD) was created as a model of nonunion that could be used by researchers to test bone replacement materials (BRM) in a consistent manner [1,3,4]. The need for such a standardized defect came from the routine practice of each researcher using their own specific surgical model, making comparison between BRM almost impossible [3]. CSDs have been used for two decades to test BRMs, cellular therapies, and other bone replacement strategies. Because CSDs are the only standard craniofacial bone defect model, it is logical to use it as a basis for the development of novel models of craniofacial bone healing.

The CSD, at its inception, was defined in terms of the size of the defect that would not heal, regardless of how much time it was given to heal [3]. CSDs were developed to model fibrous nonunions in humans. These nonunions are not capable of healing without medical assistance. Therefore, the ultimate goal for the model was to create a bone defect in animals that would be “shut down” and be unable to heal on its own, similar to human nonunions.

There is an intuitive difference between the biology of a bone defect that is healing but has not completely healed and the defect that has filled with fibrous tissue and will never heal. Clinically, the term “nonunion” is given to a defect that is not healed within 8 months of injury [23], but the decision to intervene surgically is mainly up to the individual clinician. This clinical definition that employs an 8 month “cut-off” point can be altered, based on the discomfort of the patient. It is important to notice that the definition of a CSD, which was developed to model human nonunion, is based on the size of the defect that will not heal within the lifetime of the animal. There is no direct clinical correlate to the CSD defined in this way because such a definition would depend on the size of the initial bone wound and on the outcomes measured at the time of the patient’s death.

More importantly, there seems to be no clinical relevance to the fact that CSDs will not heal. In orthopaedic reconstruction, nonunions are debrided and a new bone defect is created and treated, often by a bone autograft. At no point is the nonunion or the fibrous tissue that fills an orthopaedic nonunion treated directly. Different from the orthopaedic nonunion is nonunion following cranial vault reconstruction. In the latter case, the dura mater underlying the defect often becomes scarred and calcified. This fibrous tissue cannot be disturbed in order to avoid a dural tear. Though the translation of a defect that is “shut down” does seem to apply to the clinical reality of calvarial defects, no research has yet been performed to test therapies to directly treat the fibrous tissue within a CSD. Because of the weak clinical applicability, and the lack of experimental backing for the biological relevance, the definition and use of CSDs must be amended.

Re-defining “critical-size defects”—A few researchers have found that the original definition of CSD is not really functional. Gosain et al. stated that “a critical-size defect is one that will not heal within the lifetime of the animal. However, because most studies are of limited duration and do not extend over the entire life of the animal, the critical-size defect in animal research refers to the size of a defect that will not heal over the duration of the study” [21]. This new definition dropped the idea of “smallest interosseus defect” and the dependence on the “lifetime of the animal.”

Though this re-definition is more relevant because it is based solely on time, not on defect size, it also undermines the standardization of defects for bone healing research. The definition hinges on the length of the study, not the surgical model. Therefore, if a very small defect is made, and analysis is performed in a very short time (one hour, for example), should the small defect be considered critical-size? If the answer is yes, then there is no point to defining “critical-size defects” in order to standardize research practices because each researcher can utilize his or her own defect to test bone healing strategies.

Also, the CSD theoretically models the nonunion by simulating a defect that is “shut down” and will never heal. By changing the definition to a defect that will not heal over the course of the study, we lose the concept that the biology might be different for non-healing defects than it is for normally healing or slowly healing defects. Therefore, this re-definition may also undermine the attempt to model human nonunions.

The future of “critical-size defects”—Since “critical-size defects” were arbitrarily defined rather than experimentally generated and researchers have identified problems with the definition, there appear to be three choices regarding the future of the term: 1) the term “critical-size defect” can be used as defined by Schmitz et al. [3] if it is strengthened by analyzing the healing of defects of all sizes at the end of the animals’ lifetimes to experimentally determine the smallest defect that will not heal in the lifetime of the animal; 2) the CSD can be re-defined as any defect that does not heal over the duration of the study and have no standardization or good clinical correlate to the model; or 3) the use of the term “critical-size defect” can be discontinued.

Of these choices, the most logical appears to be to discontinue the use of the term “critical-size defect.” The model has only a limited clinical applicability and currently only serves to standardize the research methodology. However, through the use of μ CT or other in vivo imaging techniques, the size of the initial defect can more accurately be determined in live animals and the amount (volume) and quality (density, micromorphology) of the bone that is formed can be more accurately measured. Such quantifiable measurements allow each researcher to design a surgical defect model that most closely resembles their area of clinical interest. Technology is enabling the quantification of small differences in the amount and quality of bone formation within small defects. The future of bone research will lie in the development of treatment modalities that are tailored to specific clinical applications. Furthermore, it is imperative that investigators develop models that accurately reflect the clinical realities that they encounter. Specifically, it is important to model each of the factors that may influence bone healing in specific patient populations, including age, nutrition, radiation treatment, scarring, or infection. This strategy necessitates the use of appropriate animal models and technological advances and it minimizes the need for a critical-size defect model.

Acknowledgments

This work was supported in part by grants from the NIH/NIDCR (DE013420 [JH] and DE019430 [GC]).

References

1. Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. *J Craniofac Surg* 1990;1:60–68. [PubMed: 1965154]
2. Mooney, MP.; Siegel, MI. Animal models for bone tissue engineering of critical-sized defects (CSDs), bone pathologies, and orthopedic disease states. In: Hollinger, JO.; Einhorn, TA.; Doll, BA.; Sfeir, C., editors. *Bone Tissue Engineering*. Boca Raton, FL: C.R.C. Press; 2005. p. 217–244.
3. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop* 1986;299–308. [PubMed: 3084153]
4. Schmitz JP, Schwartz Z, Hollinger JO, Boyan BD. Characterization of rat calvarial nonunion defects. *Acta Anat (Basel)* 1990;138:185–192. [PubMed: 2389661]
5. Krebsbach PH, Mankani MH, Satomura K, Kuznetsov SA, Robey PG. Repair of craniotomy defects using bone marrow stromal cells. *Transplantation* 1998;66:1272–1278. [PubMed: 9846508]
6. Wang J, Glimcher MJ. Characterization of matrix-induced osteogenesis in rat calvarial bone defects: I. Differences in the cellular response to demineralized bone matrix implanted in calvarial defects and in subcutaneous sites. *Calcif Tissue Int* 1999;65:156–165. [PubMed: 10430651]
7. Mooney, MP.; Siegel, MI. Animal models for bone tissue engineering. In: Wnek, G.; Bowlin, G., editors. *Encyclopedia of Biomaterials and Biomedical Engineering*. New York: Marcel Dekker; 2005. p. 1–19.
8. Mikos AG, Herring SW, Ochareon P, Elisseeff J, Lu HH, Kandel R, Schoen FJ, Toner M, Mooney D, Atala A, et al. Engineering complex tissues. *Tissue Eng* 2006;12:3307–3339. [PubMed: 17518671]
9. Aalami OO, Nacamuli RP, Lenton KA, Cowan CM, Fang TD, Fong KD, Shi YY, Song HM, Sahar DE, Longaker MT. Applications of a mouse model of calvarial healing: differences in regenerative abilities of juveniles and adults. *Plast Reconstr Surg* 2004;114:713–720. [PubMed: 15318051]
10. Cowan CM, Shi YY, Aalami OO, Chou YF, Mari C, Thomas R, Quarto N, Contag CH, Wu B, Longaker MT. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat Biotechnol* 2004;22:560–567. [PubMed: 15077117]
11. Pryor ME, Susin C, Wikesjo UM. Validity of radiographic evaluations of bone formation in a rat calvaria osteotomy defect model. *J Clin Periodontol* 2006;33:455–460. [PubMed: 16677336]
12. Lee JY, Musgrave D, Pelinkovic D, Fukushima K, Cummins J, Usas A, Robbins P, Fu FH, Huard J. Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice. *J Bone Joint Surg Am* 2001;83-A:1032–1039. [PubMed: 11451972]
13. Babler WJ, Persing JA, Persson KM, Winn HR, Jane JA, Rodeheaver GT. Skull growth after coronal suturectomy, periosteotomy, and dural transection. *J Neurosurg* 1982;56:529–535. [PubMed: 7062124]
14. Fatah MF, Ermis I, Poole MD, Shun-Shin GA. Prevention of cranial reossification after surgical craniectomy. *J Craniofac Surg* 1992;3:170–172. [PubMed: 1298415]
15. Spector JA, Greenwald JA, Warren SM, Bouletreau PJ, Crisera FE, Mehrara BJ, Longaker MT. Co-culture of osteoblasts with immature dural cells causes an increased rate and degree of osteoblast differentiation. *Plast Reconstr Surg* 2002;109:631–642. discussion 643–644. [PubMed: 11818846]
16. Spector JA, Greenwald JA, Warren SM, Bouletreau PJ, Detch RC, Fagenholz PJ, Crisera FE, Longaker MT. Dura mater biology: autocrine and paracrine effects of fibroblast growth factor 2. *Plast Reconstr Surg* 2002;109:645–654. [PubMed: 11818848]
17. Greenwald JA, Mehrara BJ, Spector JA, Chin GS, Steinbrech DS, Saadeh PB, Luchs JS, Paccione MF, Gittes GK, Longaker MT. Biomolecular mechanisms of calvarial bone induction: immature versus mature dura mater. *Plast Reconstr Surg* 2000;105:1382–1392. [PubMed: 10744229]
18. Greenwald JA, Mehrara BJ, Spector JA, Fagenholz PJ, Saadeh PB, Steinbrech DS, Gittes GK, Longaker MT. Immature versus mature dura mater: II. Differential expression of genes important to calvarial reossification. *Plast Reconstr Surg* 2000;106:630–638. discussion 639. [PubMed: 10987470]
19. Wang J, Glimcher MJ. Characterization of matrix-induced osteogenesis in rat calvarial bone defects: II. Origins of bone-forming cells. *Calcif Tissue Int* 1999;65:486–493. [PubMed: 10594169]

20. Gosain AK, Santoro TD, Song LS, Capel CC, Sudhakar PV, Matloub HS. Osteogenesis in calvarial defects: contribution of the dura, the pericranium, and the surrounding bone in adult versus infant animals. *Plast Reconstr Surg* 2003;112:515–527. [PubMed: 12900610]
21. Gosain AK, Song L, Yu P, Mehrara BJ, Maeda CY, Gold LI, Longaker MT. Osteogenesis in cranial defects: reassessment of the concept of critical size and the expression of TGF-beta isoforms. *Plast Reconstr Surg* 2000;106:360–371. discussion 372. [PubMed: 10946935]
22. Nussenbaum B, Krebsbach PH. The role of gene therapy for craniofacial and dental tissue engineering. *Adv Drug Deliv Rev* 2006;58:577–591. [PubMed: 16766080]
23. Brinker, MR. Nonunions: Evaluation and Treatment. In: Browner, BD.; Jupiter, JB.; Levine, AM.; Trafton, PG.; Green, NE.; Swiontkowski, MF., editors. *Skeletal Trauma: Basic Science, Management, and Reconstruction*. New York: Saunders; 2003. p. 507

Figure 1.

Intraoperative photograph of defect created using the “elevator” technique. **A)** Photograph of defect created by fracturing through the endocortical layer of the parietal bone. The undamaged blood vessels within the defect (arrows) demonstrated that the dura was left intact. **B)** Same picture as in A with an outline of the endocortical defect margin. Notice that the margin is not uniform because of the fracturing technique that was used in the elevator group.

Bone Healing at 6 Weeks

Figure 2.

Analysis of untreated mouse 1.8mm calvarial defects 6 weeks postoperatively. **A)** Radiograph showing remnant defect in mouse calvaria 6 weeks after creating a 1.8mm outer diameter defect. Yellow dashed circle shows the outline of the original defect. **B)** Graph showing the 2D measurement of bone formation ($\pm$ SEM) within the defect after 6 weeks.

4 Wk Bone Healing

8 Wk Bone Healing

1 yr Bone Healing

Figure 3.

2-Dimensional radiographic analysis of surgical technique effect on defect healing. **A)** Graph showing the mean ($\pm$ SEM) area of new bone formation within defects 4 weeks after surgery. The trephine group healed approximately 26% while the elevator group healed approximately 35% after 4 weeks. **B)** Graph showing defect healing 8 weeks after surgery at which time the trephine group had healed approximately 25% compared to nearly 50% healing in the elevator group. **C)** Radiographs showing the initial defects (day 0) and healing at 1 year for all groups. **D)** Graph showing the mean ($\pm$ SEM) of defect healing in all groups 1 year after surgery. After 1 year, the trephine group only healed about 35% of each defect, and the elevator group healed approximately 52%. As shown in D, dural damage group healed similarly to the trephine group at all times (not shown).

Rat Parietal Bone Defect Healing

Figure 4.

Rat parietal bone defect healing 6 weeks postoperatively. **A)** Radiograph showing the defect remaining after 6 weeks of healing. Notice that most of the healing occurred around the perimeter with small islands of bone forming within the defect. **B)** Analysis of remaining defect area ($\pm$ SEM) determined that these defects healed approximately 37% of the original defect area (2.3mm diameter = 4.155mm² area) after 6 weeks.

Table 1

Chart showing the three different experiments used to test models of healing calvarial defects.

Species	Surgical Technique	Defect Size	Post-operative time analyzed	Sample Size
Mouse	Trephine	1.8 mm	6 weeks	10
Mouse	Trephine, Elevator, Intentional Dural Damage	1.8 mm	4 weeks, 8 weeks, 1 year	6 in each group at each time point
Rat	Trephine	2.3 mm	6 weeks	6