A Nasal Critical-Size Defect

An Experimental Model for the Evaluation of Facial Osseous Repair Techniques

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Objective: To create a standardized nonhealing defect of craniofacial, minimal load–bearing, endochondral type bone with geometric properties that are amenable to quantitative and biomechanical testing that can be used to develop new osteoconductive and osteoinductive engineering repair techniques.

Design: Before-and-after randomized trial of an anatomical description.

Subjects: Twenty-four retired male breeder Sprague-Dawley rats.

Methods: A standardized osseous defect was created by removing the nasal bones with a cutting burr to the level of the nasal mucosal membranes. The defects were not repaired, and groups of 8 animals were examined using planimetry, computed tomographic scanning, and histological analysis at 1, 3, and 6 months following surgery to quantify defect repair.

Results: Mean repair rate by surface area measurements at 1, 3, and 6 months was 5.75%, 4.89%, and 7.09%, respectively. Results from histological analysis revealed that the defects were filled with fibrous tissue. Computed tomographic scans showed the bone defect without repair.

Conclusion: This nasal osseous defect fulfills criteria to be considered as a critical-size defect that can be used to investigate new techniques for bone reconstruction.


D effects in the facial structural framework are common and challenging problems for otolaryngologist–head and neck surgeons that occur as a result of congenital malformation, trauma, infection, and the surgical resection of neoplastic disease. Current materials for repairing these defects involve autogenous bone and cartilage, allograft bone, and synthetic alloplasts. At present, however, all of these techniques have potentially serious drawbacks ranging from unpredictable resorption and migration of the implant to a second surgical site to the possibility of viral transference. The ideal method of repair has yet to be developed.

Diverse research is under way to develop improvements in facial bone reconstructive options. The use of critical-size defects (CSDs) as standardized models to evaluate osteogenic materials has been proposed by Hollinger and Kleinschmidt. The CSD is defined as an osseous defect, which, if left untreated, shows less than 10% healing of bone during the lifetime of an animal. Critical-size defects therefore provide reproducible models from which new methods of bone repair can be developed and tested. Unfortunately, no standardized and consistent model of a facial osseous defect has been described. This study proposes a novel site for a facial CSD using the nasal bone of rats as the first step in developing improved facial reconstruction techniques.

RESULTS

Precise surface area planimetry of the nasal defects showed a mean repair rate of 5.75% at 1 month, 4.89% at 3 months, and 7.09% at 6 months (Table). The overall mean repair rate for all groups was 5.91% by surface area. Only 1 rat (in the 1-month group) showed greater than 10% repair; no obvious explanation could be found for this variance. Gross and histological analysis revealed that all wounds appeared to undergo a small amount of new bone growth from the perimeter of the defect (Figures 4 and 5). After the noses were opened for examination, a thick fibrous substrate was seen in the wound fields that easily lifted away from the bone.
MATERIALS AND METHODS

Twenty-four retired male breeder Sprague-Dawley rats weighing between 550 and 650 g underwent identical surgical procedures to create dorsal nasal defects. All procedures were carried out in accordance with approved guidelines of the University of Virginia for the humane treatment of animals in medical research. After the rats received anesthesia with 17 mg/kg of intraperitoneal pentabarbital sodium and 10 mg of intramuscular ketamine hydrochloride, the hair on their noses and faces was shaved and prepared with a depilatory agent. The rats were placed on a standardized cephalostat and a midline incision was made from the nasal supratip to the forehead. Skin flaps were elevated and retracted laterally. The periosteum was incised, elevated, and retracted lateral to the maxillonasal suture lines (Figure 1). Under an operating microscope, a 3-mm cutting burr was used to remove the nasal bones down to the level of the mucosal membranes of the superior nasal vault. Care was taken not to violate the nose. A 1-mm diamond burr was used to make a 20 × 8-mm rectangular defect and to smooth the edges (Figure 2). Copious irrigation was followed by precise planimetry (Table). The periosteum was then closed with 6-0 prolene suture. The skin was closed in 1 layer with 4-0 nylon and the animals were returned to the vivarium. No special diet or activity was instituted.

At 1, 3, and 6 months following surgery, 8 animals were randomly retrieved and humanely killed. The heads were examined using a computed tomographic scanner (9800; General Electric, Milwaukee, Wis), and 3-dimensional reconstruction was performed (Figure 3). The incisions were opened and the defects were photographed and measured with precise surface area planimetry (Figure 4 and Table). The specimens were then submitted for histopathologic analysis after fixation with a 10% buffered formalin solution and decalcified with EDTA hydrochloric acid (JT Baxter Inc, Phillipsburg, NJ). The specimens were cut in an axial plane, embedded in paraffin, and stained with hematoxylin-eosin (Figure 5).

and nasal mucosal membranes. There was no evidence of infection, nasal obstruction, or collapse in any animal. Computed tomographic scans showed the dorsal nasal defects (Figure 3); however, volumetric analysis was not possible because of the limited vertical height of the defects themselves (<3 mm).

COMMENT

The rodent nasal defect created in our study fulfills criteria to be considered a CSD and therefore can be used for the development of alternative techniques of facial osseous reconstruction. Hollinger and Kleinschmidt defined the CSD as a defect that heals less than 10% during the lifetime of an animal. For practical purposes, however, the retired male breeder rats used in this experiment were 18 months and had a 2-year life expectancy. Because the nasal defects do not heal within 6 months, it is unlikely that they would ever have healed during the normal life span of the rodents. By definition, spontaneous repair of bone does not occur in a CSD. Repair of a CSD requires materials that are osteogenic. In the absence of such materials, CSDs form fibrous connective tissue, not bone, to span the defect. During autopsy, this fibrous tissue filler was identified and easily lifted out of the defect for planimetric measurement.

Our CSD provides an ideal model for the investigation of new reconstruction options. It is a non–load-bearing immobile defect between the strong medial midface buttresses and is subject to minimal force vectors or motion stressors. Our nasal CSD is also unique in that it is the first CSD to be described that derives from enchondral rather than intramembranous bone. Critical-size defects have been described in other nonfacial areas. Rat calvarial models have been described and used to evaluate new osteoconductive materials. However, these models are less than optimal for the evaluation of facial reconstruction because they are composed of membranous rather than enchondral bone, and morbidity due to brain injury and meningitis is not uncommon. The
canine mandible has been evaluated as well, yet unlike this nasal model, it is a load-bearing and mobile structure subject to a variety of uncontrollable stressors. The mandible is also of complex embryological origin, which makes bone repair more difficult to evaluate than the nasal model. Long-bone CSDs have also been described, but they have the significant drawback that the animal must either be restrained or have an external brace to limit strain in the defect while osteogenic interventions are tested. Infection has also been reported as a consequence of this rigid fixation.

This rat nasal CSD model has other practical advantages as well. In the rodent, this defect is easily reproducible. Mature male animals were used in this study to avoid both the increased osseous repair of immature animals and the estrous cycle of female animals. No extrapolation of these results is possible for juvenile or female rodents without further testing. Other advantages of using retired breeders, aside from probable age-related reductions in bone healing rates, are cost (approximately the cost of 2 normal adult rats), maximal use of animals that have been used for reproductive purposes, and nasal bones larger than those of young 450- to 500-g male rats that are usually used experimentally. This size requirement appears to be critical to the success of the model. During subsequent experiments, similar-sized retired male breeder rats ordered from the same

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<th>Postoperative Month</th>
<th>Animal No.</th>
<th>Initial Defect, cm²</th>
<th>Necropsy, cm²</th>
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*Mean healing for all groups was 5.91%.
supplier appeared to have smaller midfacial anatomical features; as a result, we had to place multiple orders for animals. Work is currently under way to confirm CSD status with a smaller defect on smaller retired breeder rats. Additionally, the procedure appears to have no adverse effects on the rodents’ respiration or mastication. No infections were observed through the course of developing this model and the experiment or in subsequent osteoengineering trials. This includes several occasions in which the nasal mucosal membranes were perforated when thick gelatinous implants were placed into the defect. This CSD model has been successfully used to test osteoconductive and osteoinductive repair techniques in experiments in which up to 100% repair was attained with collagen implants under optimal conditions, compared with less than 7% healing in controls.\textsuperscript{11,12}

The nose is also conducive to quantifying repair. Although computed tomographic scanning was used in this model’s development and in other studies, its contribution in terms of practical knowledge gained is limited. This is because of the shallow (<3 mm) depth of the defect and the difficulty in placing the rodent’s head in precise alignment with the scanner, which has a limited resolution at such small dimensions. However, by manipulating the scan with 3-dimensional software, it is possible to achieve virtually any result desired. A newer technique that we are currently exploring involves the use of a standard planar x-ray film of the excised dorsum, which is scanned into a computer for radiodensitometric analysis. Early results from ongoing research suggest that this technique is more reliable, less expensive, and less apt to produce aberrant data than computed tomographic scanning.\textsuperscript{12} Finally, the geometric configuration of this nasal model lends itself to strength and torsional quantification if indicated.

**CONCLUSIONS**

The reproducible nasal osseous defect we report herein heals less than 10% by surface area over a 6-month period without intervention and fulfills criteria to be considered a CSD. Its unique location on the central face in endochondral bone allows it to be used as a model in which new techniques for osseous repair can be evaluated.

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**REFERENCES**

10. Sweeney TM, Chabra A, Opperman LA, Persing JA, Ogle RC. Repair of rat nasal critical size defects using insulin-like growth factor-1 augmented type I collagen gels. La-

**REFERENCES**

11. Tournig J, Griffin AA, Ogle RC, Lindsey WH. Repair of rat nasal critical sized defects using insulin like growth factor-1 augmented type I collagen gels. La-

**REFERENCES**