INTRODUCTION
The establishment of new therapeutic approaches for tissue repair and regeneration can be accelerated if adequate technologies for monitoring the repair process are developed. In craniofacial surgery, 96,000 bone graft procedures are performed each year to treat bone loss due to trauma or disease. Alas, autologous bone grafts are not always available, and additional surgery must be performed for its harvest. Alternatively, there is a large potential supply of craniofacial allografts composed of nonvital bone. However, these grafts often fail to integrate with host bone due to formation of scar tissue. Recently, it was shown that daily teriparatide (recombinant human parathyroid hormone, PTH) treatment enhances integration of devitalized allograft in long bones and inhibits scar formation. We hypothesized that PTH treatment would enhance allografts in cranial membranous bones as well; furthermore, we postulated that this bone healing process could be monitored using functional fluorescence imaging (FLI) in vivo.

Figure 1: Fluorescence imaging on Day 21 (A). Quantification of fluorescence efficiency (total, average, and maximum) was performed in the marked standard region of interest (ROI) on Days 7 (B), 14 (C), and 21 (D). *p < 0.05; bars represent ± SE; Blue bars = Autograft group; red bars = Allograft group (no PTH); green bars = Allograft+PTH group.

METHODS
Circular calvarial bone defects (5 mm in diameter) were created in FVB/n mice. The mice were then divided into 3 groups and given implants of allografts, with or without daily PTH treatment (40 µg/kg/day), or autografts (n = 5 per group). In vivo FLI of the bone formation process was performed on Days 7, 14, and 21 postimplantation. The mice were imaged to detect fluorescence by using an IVIS® Kinetic system (Caliper Life Sciences, Hopkinton, MA). Osteoblast activity (bone formation) was imaged using the fluorescent probe OsteoSense9 680/800 (PerkinElmer Inc., Waltham, MA), a hydroxypatite-directed bone-imaging probe, which was systematically administered. Bone formation was also analyzed using a micro-computed tomography (micro-CT) scanner (µCT 40; Scanco Medical AG, Brüttisellen, Switzerland), which was set at a nominal resolution of 16 µm. To further validate the imaging data, RNA was isolated from another set of mice calvariae, reverse transcription was performed, and qPCR was used to evaluate the expression of the following osteogenic genes: alkaline phosphatase (ALP), osrix (Ocx), osteocalcin (Oc), osteopontin (OPN), and bone sialoprotein (Bsp). Statistical analysis was performed using a two-tailed homoscedastic Student t-test.

RESULTS
Fluorescence imaging of bone formation in the allograft+PTH group showed a significantly higher fluorescent signal on Day 7 and Day 21 when compared to the allograft group not treated with PTH, and on Day 14 when compared to the autograft group (Fig. 1). MicroCT analysis showed that the healing process was not completed by Day 21 postimplantation, yet significantly more bone was formed in the allograft+PTH group than in the autograft group (Fig. 2). Significantly higher levels of expression of the osteogenic genes Oc and Bsp (on Days 10 and 14) were found in the allograft+PTH group in comparison with the group treated with an allograft but no PTH. No significant differences were found in the other osteogenic genes that we tested.

Figure 2: MicroCT imaging on Day 21. Quantification of bone volume was performed in a standard 5-mm-diameter cylinder volume of interest, highlighted in yellow.

DISCUSSION
The longitudinal, noninvasive FLI system allowed us to follow the effect of the combined PTH-Allograft treatment on the repair process in calvarial bone defects. Results from another ongoing study in long-bone grafts treated with PTH suggest that the effect of PTH may be mediated by changes in vasculogenesis. We are currently investigating this hypothesis over a longer period by using functional FLI and additional fluorescent probes for monitoring angiogenesis. The imaging approach described in this work is an initial and novel step in the process of close follow-up of the bone repair process and could also be applied for other anatomical sites as well.

SIGNIFICANCE
Results from this study and other ongoing experiments are designed to provide preclinical efficacy data to support a clinical trial of PTH-allograft combined therapy for Bone Repair in adult patients suffering from calvaria trauma. Thus this program has potential for immediate translation and a significant impact.

ACKNOWLEDGMENT
We acknowledge funding from the National Institutes of Health (NIDCR DE019902).