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 craniomaxillofacial 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 induces integration of allografts also in cranial membranous bones via several mechanisms as depicted on Diagram 1: 1. Enhanced homing of MSCs to the site of injury; 2. Enhanced differentiation of MSCs to osteoprogenitors; 3. Modulation of the vasculogenesis in the defect proximity decreases the abundance of mast cells, thus leading to more efficient bone formation on the expanse of fibrous tissue.

Methods:

To pursue this hypothesis, we created 5-mm-diameter calvarial bone defects in transgenic FVB/N mice, which express luciferase under the control of the osteocalcin promoter. 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 bioluminescence imaging (BLI) was performed to monitor host osteoprogenitor differentiation at the implantation site. In vivo fluorescence imaging (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 (i.e., bone formation) was imaged using the fluorescent probe OsteoSense™ 680/800 (PerkinElmer Inc., Waltham, MA), a hydroxyapatite-directed bone-imaging probe, which was systemically administered. Bone formation and vasculogenesis was also analyzed using a micro-computed tomography scanner (μCT 40; Scanco Medical AG, Brütisellen, 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), osterix (Osx), osteocalcin (Oc), osteopontin (OPN), and bone sialoprotein (Ibsp). Statistical analysis was performed using a two-tailed
Endogenous osteoprogenitor cells were detected using immunofluorescence (IF) staining against the MSC surface markers CD90 (ab3105, Abcam, Cambridge, MA), CD44 (ab25340), and CD29 (ab52971) by using primary anti-mouse antibodies and secondary antibodies conjugated to AlexaFluor® 488 (Invitrogen) or Cy3 (Jackson ImmunoResearch, West Grove, PA). Mast cells were detected by performing IF against two mast cell markers: mast cell protease 1 (MCP1, MAB5146, R&D Systems, Minneapolis, MN) and mast cell tryptase (MCT, LS-C18207, LifeSpan Biosciences, Inc., Seattle, WA) and using toluidine blue staining.

Results:
Osteoprogenitor differentiation, as detected by BLI, in mice treated with an allograft implant and PTH was over 2-fold higher than those in mice treated with an allograft implant without PTH (Fig. 1). FLI also demonstrated that the bone mineralization process in PTH-treated allografts was significantly higher than that in untreated allografts (Fig. 2). The μCT scans revealed a significant increase in bone formation in Allograft + PTH-treated mice compared to Allograft + PBS treated mice (Fig. 3). The histological analysis showed enhanced bone formation around the allograft and decreased fibrotic tissue formation in the PTH-treated animals (Fig 4). The osteogenic genes osteocalcin (Oc/Bglap) and integrin binding sialoprotein (Ibsp) were upregulated in the Allograft + PTH-treated animals (data not shown). We found that PTH modulated the infiltration of mast cells to the allograft proximity (Fig. 5).

Figure 1. PTH treatment enhanced host osteoprogenitor cell differentiation, as detected by bioluminescence imaging (BLI). Allogeneic calvarial bone grafts were implanted in mice expressing luciferase driven by the osteocalcin promoter. Half of the mice were treated with daily subcutaneous injections of PTH and the other half with injections of PBS (mock treatment). At designated time points, the mice were systemically injected with luciferin substrate and BLI was performed. The BLI signal was normalized to each animal’s tail. For use as a control, we imaged mice that had undergone a sham procedure (no defect) and were given PTH or PBS (mock treatment); n = 8, bars represent ± SE; * p < 0.05 (Allograft + PTH vs. all other groups).
Figure 2. PTH enhanced bone mineralization, as quantitatively detected by functional fluorescence imaging (FLI): Six days after surgery, mice were treated with PTH or PBS, were injected with a hydroxyapatite-directed imaging probe, OsteoSense680™. n = 5, bars represent ± SE; * p < 0.05.
Figure 3. PTH therapy enhanced bone formation in a calvarial bone defect: \(\mu\)CT quantitative analysis in vivo. Devitalized allografts were implanted in calvarial bone defects with SQ injections of PTH or PBS for 3 weeks. Longitudinal \(\mu\)CT analysis over time showed a significant elevation in bone formation in the PTH-treated group as early as Week 4. The bone volume was normalized to that seen on Day 1 scans. Bars represent \(\pm SE\), \(*p < 0.05\), \(n = 7\).

Figure 4. PTH promoted the repair of a calvarial bone defect by decreasing scar tissue formation: histological analysis. Standard hematoxylin and eosin (H&E) staining shows excellent integration of the allograft into host bone tissues with minimal fibrous tissue formation between the graft and host bone. AG - allograft. F - fibrous tissue. H - host bone, NB - new bone formation.
Figure 5. A porcine model for allograft-based craniofacial bone regeneration: Segmental bone defect was created in a pig mandible (3D reconstruction of cone-beam computed tomography scan, a). In order to enable allograft integration and bone healing while reducing the risk of infection from the oral cavity, an acrylic space-maintainer was affixed into the defect to allow gingival healing prior to allograft implantation (b).

Discussion:
We found that PTH treatment enhances osteoprogenitor differentiation and augments bone formation around structural allografts. The precise mechanism is not clear, but we show that infiltration pattern of mast cells, associated with the formation of fibrotic tissue, in the defect site is significantly affected by the PTH treatment.

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-related trauma. Thus, this study has the potential for immediate translation resulting in significant clinical impact.

Acknowledgments:
We acknowledge funding from the National Institutes of Health (NIDCR DEO19902).

ORS 2014 Annual Meeting
Poster No: 0016