J Clin Periodontol 2010; 37: 419–426 doi: 10.1111/j.1600-051X.2010.01547.x

Journal of Clinical Periodontology

Effect of systemic parathyroid hormone (1–34) and a β-tricalcium phosphate biomaterial on local bone formation in a critical-size rat calvarial defect model

Jonathan I. Yun[1], Ulf ME Wikesjö[2], James L. Borke[3], Frederick C. Bisch[1], Jill E. Lewis[3], Robert W. Herold[1], Gary D. Swiec[4], Joseph C. Wood[5] and James C. McPherson III[5]

[1]US Army Advanced Education Program in Periodontics, US Army, Ft. Gordon, GA, USA; [2]Laboratory for Applied Periodontal & Craniofacial Regeneration (LAPCR), Departments of Periodontics and Oral Biology, Medical College of Georgia School of Dentistry, Augusta, GA, USA; [3]Department of Oral Biology, Medical College of Georgia School of Dentistry, Augusta, GA, USA; [4]US Army DENTAC, US Army, Ft. Meade, MD, USA; [5]Department of Clinical Investigations, US Army, Ft. Gordon, GA, USA

Yun JI, Wikesjö UME, Borke JL, Bisch FC, Lewis JE, Herold RW, Swiec GD, Wood JC, McPherson III JC. Effect of systemic parathyroid hormone (1–34) and a β-tricalcium phosphate biomaterial on local bone formation in a critical-size rat calvarial defect model. J Clin Periodontol 2010; 37: 419–426. doi: 10.1111/j.1600-051X.2010.01547.x.

Abstract

Objective: The objective of this study was to evaluate local bone formation following systemic administration of parathyroid hormone (1–34) (PTH), a surgically implanted synthetic β-tricalcium phosphate (β-TCP) bone biomaterial serving as a matrix to support new bone formation.

Materials and Methods: Critical-size, 8 mm, calvarial through-and-through osteotomy defects were surgically created in 100 adult male Sprague–Dawley rats. The animals were randomized into five groups of 20 animals each to receive one of the following treatments: PTH (15 μg PTH/kg/day; subcutaneously), PTH/β-TCP, β-TCP, or particulate human demineralized freeze-dried bone (DFDB), and sham-surgery controls. Ten animals/group were euthanized at 4 and 8 weeks post-surgery for radiographic and histometric analysis.

Results: The histometric analysis showed that systemic PTH significantly enhanced local bone formation, bone fill averaging (± SE) 32.2 ± 4.0% compared with PTH/β-TCP (15.7 ± 2.4%), β-TCP (12.5 ± 2.3%), DFDB (14.5 ± 2.3%), and sham-surgery control (10.0 ± 1.5%) at 4 weeks ($p < 0.014$). Systemic PTH showed significantly enhanced bone formation (41.5 ± 4.0%) compared with PTH/β-TCP (22.4 ± 3.0%), β-TCP (21.3 ± 4.4%), and with the sham-surgery control (23.8 ± 4.2%) at 8 weeks ($p < 0.025$). The DFDB group showed significantly increased bone formation from 4 (14.5 ± 2.3%) to 8 weeks (32.0 ± 3.2%) ($p < 0.006$). The PTH/β-TCP and β-TCP groups both showed limited biomaterials resorption. The radiographic analysis was not diagnostic to distinguish local bone formation from the radiopaque β-TCP biomaterial.

Conclusions: Systemic administration of PTH significantly stimulates local bone formation. Bone formation was significantly limited by the β-TCP biomaterial.

Key words: β-tricalcium phosphate; guided bone regeneration; parathyroid hormone; rat calvaria model; tissue engineering

Accepted for publication 20 December 2009

Conventional clinical methods to regenerate alveolar bone defects may be associated with donor site morbidity following harvesting of bone grafts or potential antigenicity and/or disease transmission following use of allogeneic or xenogeneic bone biomaterials, if at all meeting patient approval for elective procedures (Precheur 2007). Use of synthetic ceramic or polymeric bone biomaterials may eliminate such concerns. Synthetic biomaterials are suggested to serve as scaffolds for bone formation, to maintain space for bone growth, and to promote wound healing by stabilizing blood clots (Shiratori et al. 2005). Nevertheless, bone biomaterials as stand-alone therapeutic adjuncts, in general, have a

Conflict of interest and source of funding statement

The authors report no conflict of interest. The study was funded in its entirety by the USA DENTAC, Fort Gordon, GA.

limited potential to support bone formation but merely serve as fillers in support of tissue contour (Precheur 2007). As of late, however, synthetic bone biomaterials, in particular granular β-tricalcium phosphate (β-TCP) biomaterials, have been used in combination products including matrix, growth, and differentiation factors intended for periodontal regeneration and alveolar augmentation/osseointegration (Pöhling et al. 2006).

Parathyroid hormone (PTH) is an 84 amino acid regulator of calcium and phosphate metabolism. The active component consists of 34 amino acids from the N-terminus known as teriparatide (Deal & Gideon 2003, Madore et al. 2004). Although continuous exposure to PTH typically increases osteoclast density and activity and associated bone resorption, intermittent (i.e., once daily) exposure stimulates osteoblasts and results in increased bone formation in rats and humans (Skripitz & Aspenberg 2004). Intermittent administration is required for bone anabolism due to the self-limiting nature of PTH-induced survival signalling. In young rats, PTH is thought to increase differentiation of osteoprogenitor cells into osteoblasts (Skripitz & Aspenberg 2004). Osteoclast responses to PTH are most likely mediated by osteoblast activity, as PTH receptors are found on osteoblast membranes. Several mechanisms have been proposed as the mechanism for PTH, the first of which involves osteoblasts and their immature precursors signalling bone remodelling by secreting receptor activator of nuclear factor-κB ligand (RANKL). If RANKL binds to receptor activator of RANK located on the surface of osteoclasts, it promotes cell differentiation and maturation of immature osteoclasts prolonging their survival. Osteoblasts also secrete osteoprotegrin, a truncated form of RANK that is not attached to a cell membrane. Osteoprotegrin effectively binds to RANKL blocking its function and regulating bone remodelling (Deal & Gideon 2003). Histometric studies in mice have shown that a 4-week administration of PTH inhibits osteoblast apoptosis, resulting in prolonged/enhanced bone formation (Jilka et al. 1999). It has been suggested that PTH suppresses apoptosis by increasing proteins including B-cell lymphoma 2 needed for cell survival while inactivating proteins needed for apoptosis (Marx 2004). It has also been suggested that PTH stimulates insulin growth factor-1 known to increase cell proliferation and decrease apoptosis (Marx 2004, Kuemmerle 2005). Moreover, PTH increases osteoblast and osteoclast function (stimulating osteoblasts more than osteoclasts), turnover, and remodelling resulting in increased trabecular and cortical thickness and ultimately overall bone density and bone mass (Deal & Gideon 2003). Because PTH is anabolic in nature and produces osteoid at an accelerated rate, bone formation may continue to increase bone mass even after PTH has been discontinued as large amounts of osteoid are left unmineralized (Deal & Gideon 2003).

It is important to qualify that PTH stimulates osteogenesis, but does not induce de novo bone formation. Thus PTH administration should not be expected to result in complete closure of non-unions (Skripitz & Aspenberg 2004) and may benefit from the use of an osteoconductive matrix. The objective of this study was to evaluate local bone formation following systemic administration of PTH, a surgically implanted synthetic β-TCP bone biomaterial serving as a matrix for new bone formation.

Materials and Methods

Animals

One-hundred male Sprague–Dawley outbred rats (*Rattus norvegicus*), age 11–13 weeks, weight 325–375 g, obtained from a USDA-approved breeder were used. This study was conducted under an approved protocol, and animal care was delivered in accordance with guidelines established by the Institutional Animal Care and Use Committee, Dwight David Eisenhower Army Medical Center, Fort Gordon, Georgia (DDEAMC protocol 06-40A). The animals were acclimatized for 7 days; they were individually housed in plastic cages labelled with cards identifying study number, species, cage number, and animal ID. The cages were housed in purpose-designed rooms air-conditioned with 10–15 air-changes/h. Temperature and relative humidity, monitored daily, was 18–22°C and 30–70%. A 12/12 h light/dark cycle was applied. The animals had ad libitum access to water and a standard laboratory diet.

Agents and biomaterials

Rat PTH (1–34) (Sigma Chemical Corporation, St. Louis, MO, USA) in a 0.5% acetic acid solution [15 μg/kg/day; subcutaneously (s.c.)] was used to stimulate osteogenic bone formation. Synthetic, >99% pure phase β-TCP (Cerasorb®, Curasan AG, Kleinostheim, Germany) was used to serve as a matrix for PTH (1–34) stimulated bone formation. A particulate human demineralized freeze-dried bone (DFDB) biomaterial (LifeNet, Virginia Beach, VA, USA) was used to serve as a benchmark control. A polytetrafluoroethylene (PTFE) barrier (Millipore Corporation, Bedford, MA, USA) was used to contain the experimental defects.

Experimental design

The animals were randomized into five groups of 20 animals each to receive one of the following treatments: PTH, PTH/β-TCP, β-TCP, or DFDB, and sham-surgery controls. Each group was subdivided into groups of 10 to provide radiographic and histologic observations at 4 and 8 weeks post-surgery.

Surgical procedures

The animals were pre-medicated using buprenorphine HCl (0.03–0.04 mg/kg; s.c.). Anaesthesia was induced using a ketamine HCl (3.2 ml; 100 mg/ml)/xylazine HCl (0.8 ml; 20 mg/ml) cocktail (1 ml/kg; intraperitoneally (i.p.)). After induction, the skull of the animal was shaved and disinfected using a 2% chlorhexidine solution. Animals were ear-tagged, stabilized into a stereotaxic device (Stoelting Company, Wood Dale, IL, USA) fitted with an anaesthesia nose cone, and draped. Isoflurane (0.5–2.0%/O_2) was administered to maintain anaesthesia.

A periodontal surgeon (J. I. Y.) performed all surgeries in a laboratory animal surgical suite. Using aseptic routines, a 3 cm incision was made through the skin and periosteum and full-thickness flaps were reflected. Under copious sterile saline irrigation, an 8 mm through-and-through osteotomy defect was created within the calvarium using an 8 mm diamond grit trephine bur (Continental Diamond Tool Corporation, New Haven, IN, USA; Fig. 1). A pre-cut 9 mm PTFE barrier was placed over the exposed dura mater. The biomaterials (β-TCP or DFDB), as applicable, were placed over the barrier to the level of the external contour of the cranium and the defect site was covered using a 10 mm PTFE barrier. Finally, the periosteum was repositioned to cover the barrier and sutured using 4-0 chromic gut sutures (Ethicon

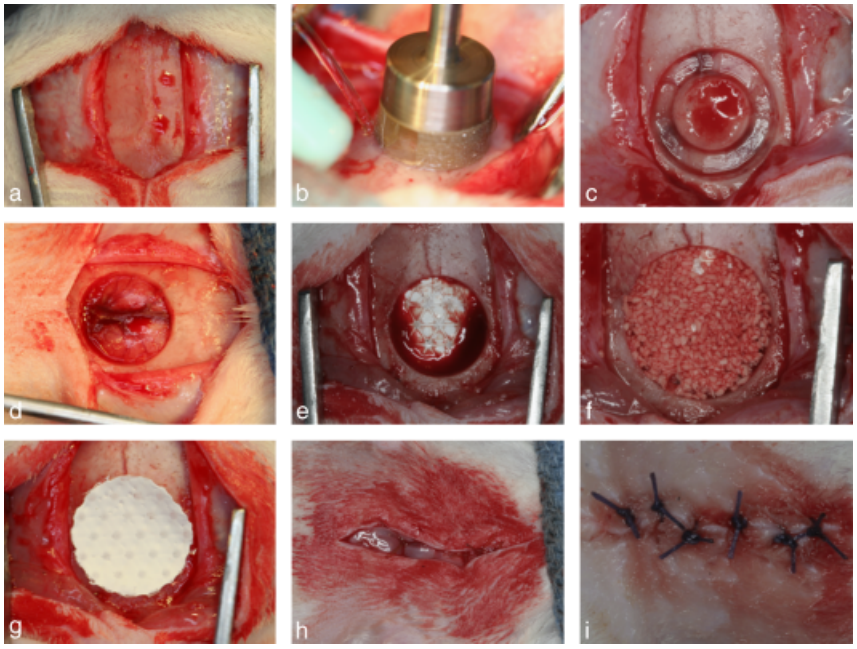

Fig. 1. Rat calvarial critical-size osteotomy defect surgery. (a) A 3 cm incision was made through the skin and periosteum. (b and c) Using lambdoidal and sagittal sutures as landmarks, 8 mm critical-size defects were made using an 8 mm diamond-coated trephine bur. (d and e) Upon removal of the bone, a 9 mm polytetrafluoroethylene (PTFE) barrier was placed ectodurally. (f) Designated biomaterials (*β*-tricalcium phosphate in this diagram) were placed into the defect. (g) 10 mm PTFE barrier was placed ectocranially. (h) The periosteum was sutured using 4-0 chromic gut. (i) Skin closure was achieved using 4-0 polyglactin sutures ensuring everted margins.

Inc. Somerville, NJ, USA), and the skin was closed using 4-0 polyglactin 910 sutures (Ethicon Inc.) ensuring everted wound margins.

Post-surgery procedures

The animals were placed in cages warmed on a circulating water heating pad and were observed for distress until they were able to move about normally. Yohimbine HCl (1–2 mg/kg; i.p.), a xylazine reversal agent, was administered to accelerate recovery as judged necessary. Buprenorphine HCl (0.02–0.03 mg/kg; s.c.) was administered every 8–12 h as needed for pain control.

PTH in a 0.5% acetic acid solution (15 μg PTH/kg/day; s.c.) was administered to animals scheduled to receive this treatment. The animals were weighed weekly and the PTH dose adjusted accordingly. Injections were scheduled to maintain consistency in daily PTH level fluctuations. Animals not scheduled to receive PTH, received 0.5% acetic acid in a saline solution as a control.

The animals were euthanized at 4 or 8 weeks post-surgery using CO_2 asphyxiation. The calvariae were harvested and fixed in 10% buffered formalin solution, soft tissues removed. Block biopsies were placed in Petri dishes and radiographed using a digital soft tissue-imaging instrument (Faxitron X-Ray, Wheeling, IL, USA).

Radiographic analysis

The average radiographic density of each defect was measured and normalized to the average density of a representative piece of cranium in uniform shape and area. After image calibration to ensure uniformity, radiographic bone fill was calculated as a percentage of the total defect area using a digital imaging software (Image J, National Institutes of Health, Bethesda, MD, USA).

Histotechnical preparation

The calvariae were sectioned for histometric analysis parallel to the sagittal suture producing a section through the center of the defect. Specimens were demineralized utilizing a decalcifying solution (Cal-Ex Decalcifying Solution, Fisher Scientific, Pittsburgh, PA, USA). The demineralized specimens were placed in cassettes, embedded in paraffin, sectioned at 3 μm in thickness using a 2030 microtome (BioCut, Leica, Reichert-Jung, Nussloch, Germany). Two central sections per defect were stained for analysis. One section was stained using haematoxylin & eosin (for the histologic and histometric analysis) and the other with Mason's blue trichrome (for the histologic analysis only).

Histometric analysis

The histologic slides were scanned to create montages using a PC-based imaging system software (Simple PCI, Hamamatsu Corporation, Sewickley, PA, USA) and a digital camera (Nikon Diaphot 300, Southern Micro Instruments, Atlanta, GA, USA) attached to an inverted microscope (Photometrics Coolsnap Fx, Roper Scientific Inc., Tucson, AZ, USA) equipped with a $\times 4$ objective lens. The area of new bone formation was measured in pixels using the digital imaging software (Adobe Photoshop; Adobe Systems Inc., San Jose, CA, USA) and expressed as a percentage of the total defect area using the haematoxylin & eosin-stained sections.

Statistical analysis

Percent bone fill and bone formation (mean $\pm$ SE) were used to compare the five groups using a one-way analysis of variance. Tukey's post hoc test was used to determine differences between groups. A p-value < 0.05 was considered significant.

Results

Clinical observations

Six animals died due to anaesthesia complications. Thus, two animals in the 4-week, three animals in the 8-week sham-surgery control, and one animal in the 4-week *β*-TCP group were lost to analysis.

Radiographic observations

Representative radiographs from week 4 and 8 are shown in Fig. 2. The sham-surgery control and sites receiving DFDB showed limited bone formation along the osteotomy perimeter at week 4. In contrast, sites receiving PTH without an adjunctive biomaterial showed significant bone formation. The sham-surgery control showed significantly

greater bone formation at week 8 compared with week 4. Similarly, bone formation significantly improved from week 4 to 8 in sites receiving DFDB or PTH without an adjunctive biomaterial. The radiopacity of the β-TCP biomaterial precluded meaningful evaluation of bone formation in all sites receiving this treatment, the radiographic appearance of the defect sites being virtually the same at week 4 and 8.

Figure 3 shows the results of the radiographic analysis. At 4 weeks, the PTH group exhibited significantly greater bone fill ($45.7 \pm 2.2\%$) than the DFDB ($28.1 \pm 1.0\%$) and the sham-surgery control ($22.9 \pm 2.8\%$) groups ($p < 0.001$). The radiopacity for sites receiving PTH/β-TCP and β-TCP amounted to ($71.0 \pm 2.4\%$) and ($73.4 \pm 2.0\%$), respectively. At 8 weeks, the PTH group exhibited significantly greater bone fill ($59.2 \pm 2.7\%$) than the sham-surgery control ($44.8 \pm 2.3\%$; $p < 0.001$) but not the DFDB group. The DFDB group showed greater bone fill at 8 ($48.5 \pm 2.9\%$) than at 4 ($28.1 \pm 1.0\%$) weeks ($p < 0.001$). The radiopacity for sites receiving PTH/β-TCP and β-TCP averaged ($70.6 \pm 3.1\%$) and ($70.6 \pm 4.4\%$), respectively.

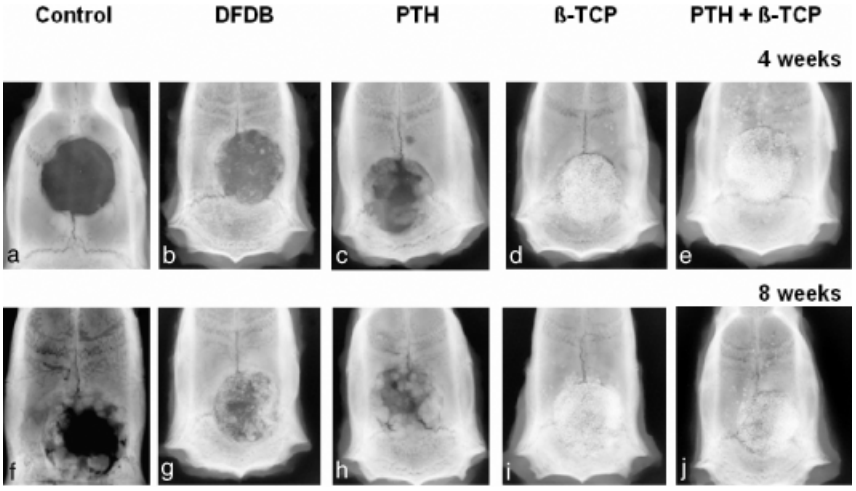

Fig. 2. Representative radiographs from the 4-week (a–e) and 8-week (f–j) observation intervals. There is significant radiopacity (bone formation) in the polytetrafluoroethylene (PTH) group at 4 weeks. There are also considerable increases in bone formation between 4 and 8 weeks for the demineralized freeze-dried bone and PTH groups compared with the sham-surgery control. The radiographs are not diagnostic for the β-tricalcium phosphate (β-TCP) and PTH/β-TCP groups due to the radiopacity of the β-TCP biomaterial.

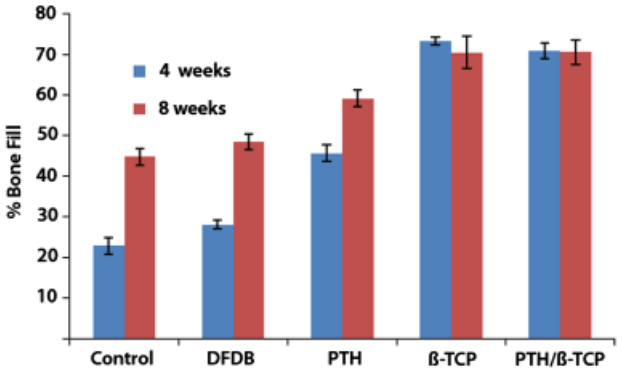

Fig. 3. Radiographic bone fill (means $\pm$ SE in %). At 4 weeks, the polytetrafluoroethylene (PTH) group exhibited significantly greater bone fill compared with the demineralized freeze-dried bone (DFDB) and sham-surgery control groups ($p < 0.001$). At 8 weeks, the PTH group showed significantly greater bone fill compared with the sham-surgery control ($p < 0.001$). The DFDB group showed greater bone fill at 8 than at 4 weeks ($p < 0.001$). The β-tricalcium phosphate (β-TCP) and PTH/β-TCP groups exhibited significantly greater radiopacity than all other groups due to presence of the radiopaque β-TCP biomaterial.

Histologic and histometric observations

Figures 4 and 5 show representative photomicrographs of the defect sites following 4- and 8-week healing intervals. At 4 weeks the PTH group showed significant bone formation throughout the defect. The sham-surgery control showed bone formation limited to the defect perimeter; the majority of the defect filled with fibrous connective tissue in addition to varying degrees of a collapsing PTFE device. Defect sites receiving β-TCP also exhibited bone formation limited to the defect perimeter with minimal bone formation amongst the β-TCP granules. The majority of the β-TCP granules remained apparently unaltered, effectively preventing collapse of the PTFE membrane into the defect site. Defect sites receiving DFDB similarly showed bone formation limited to the defect perimeter. A large portion of the DFDB particles remained and as a result effectively maintained the defect space. Similar observations were made comparing treatment groups at 8 weeks. While all groups trended towards increased bone formation, the PTH/β-TCP and β-TCP groups still showed limited biomaterials resorption and bone formation.

Figure 6 shows the results of the histometric analysis. At 4 weeks, the PTH group exhibited significantly greater bone fill ($32.2 \pm 4.0\%$) compared with the PTH/β-TCP ($15.7 \pm 2.4\%$), β-TCP ($12.5 \pm 2.3\%$), DFDB ($14.5 \pm 2.3\%$), and the sham-surgery control ($10.0 \pm 1.5\%$) groups ($p < 0.014$). At 8 weeks, the PTH ($41.5 \pm 4.0\%$) group showed significantly greater bone fill compared with the PTH/β-TCP ($22.4 \pm 3.0\%$), β-TCP ($21.3 \pm 4.4\%$), and sham-surgery control ($23.8 \pm 4.2\%$) groups ($p < 0.025$). The DFDB group however showed a significant increase in bone formation at 8 weeks ($32.0 \pm 3.2\%$) compared with that at 4 weeks ($14.5 \pm 2.3\%$) ($p < 0.006$).

Discussion

This study used a modification of a well-established rodent screening model (Schmitz & Hollinger 1986) to evaluate the osteoconductive effect of systemic administration of PTH and that of a β-TCP biomaterial on local bone formation. PTFE barriers were placed to separate the 8 mm diameter cranial

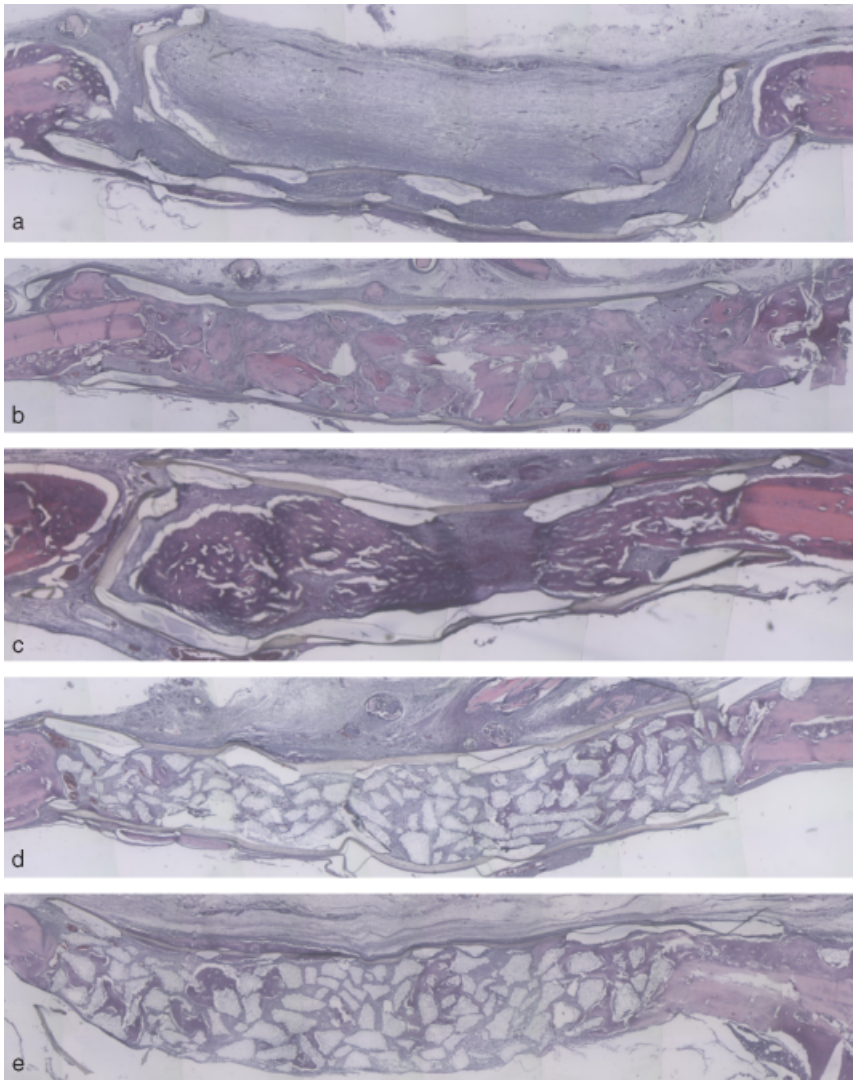

Fig. 4. Representative photomicrographs showing (a) sham-surgery control, (b) demineralized freeze-dried bone (DFDB), (c) polytetrafluoroethylene (PTH), (d) β-tricalcium phosphate (β-TCP), and (e) PTH/β-TCP specimens following a 4-week healing interval. Note extensive new bone formation in the PTH specimen. The DFDB, β-TCP, and PTH/β-TCP specimens are obturated by biomaterials showing limited, if any, appreciable bone formation (haematoxylin & eosin).

osteotomy defect from endosteal and periosteal tissues. This modification precludes immediate comparisons to other studies using the defect model without the added PTFE barriers providing baseline conditions for guided tissue regeneration (Matzenbacher et al. 2003). Nevertheless, the modified defect model exhibited baseline data of a discriminating critical-size defect model; i.e. a defect that will not fill with bone following sham-surgery procedures within the experimental lifetime of the animal, in this study 4 and 8 weeks. The rat calvaria through-and-through osteotomy defect model appears a preferred platform to screen candidate osteoconductive and osteoinductive regenerative technologies whether based on devices, biomaterials serving as osteoconductive scaffolds, biologics including matrix, growth and differentiation factors, or combinations thereof before pivotal evaluation in discriminating well-characterized large animal models and ultimately clinical settings (Schmitz & Hollinger 1986, Isaksson & Alberius 1992). The rat calvaria shares similarities with the human mandible as both develop through intramembraneous bone formation and both exhibit limited innate regenerative potential (Prolo et al. 1982). Moreover, the thin rat calvaria allows high-resolution radiographic analysis and rapid processing for histometric evaluation (Schmitz et al. 1990).

Several studies using the 8 mm critical-size rat calvaria osteotomy defect model support the practice of 4- and 8-week observation intervals. For example, Lim et al. (2000) evaluating defect fill following surgical implantation of DFDB and a resorbable membrane showed significant differences between week 1, 2, 4, and 8 in comparison with sham-surgery control. Matzenbacher et al. (2003) showed significantly greater histometric bone fill using DFDB alone or a DFDB/glycerol combination compared with glycerol alone and sham-surgery control at 8 weeks. Pang et al. (2004) compared dose-dependent responses to recombinant human bone morphogenetic protein-4 (rhBMP-4) using two carrier systems, an absorbable collagen sponge (ACS) and β-TCP at 2- and 8-week observations. The rhBMP-4/β-TCP group showed significantly greater new bone area, while the rhBMP-4/ACS group showed significantly greater bone density at 8 weeks. Ahn et al. (2003) found statistically greater histometric bone fill comparing rhBMP-4/β-TCP with controls at 2 and 8 weeks. The present study using 4- and 8-week healing intervals demonstrated accelerated bone formation following systemic administration of PTH and delayed, however, significantly enhanced bone formation following surgical implantation of DFDB.

Effects of systemic PTH administration herein corroborate previous observations showing accelerated and increased bone formation (Skripitz et al. 2000, Andreassen & Cacciafesta 2004, Alkhiary et al. 2005). At 4 weeks the histometric analysis displayed significantly greater local bone formation in animals receiving PTH as a stand-alone treatment compared with all other treatments also including the PTH/β-TCP combination; at 8 weeks animals receiving PTH solo showed significantly greater local bone formation than all other treatments but the DFDB biomaterial. Systemic PTH has been used in the management of bone diseases including osteoporosis since 2002 (Quattrochi & Kourlas 2004). A daily dose of 20 μg PTH represents the current FDA approved regimen for men and women at high fracture risk (Cosman 2005). PTH enhances bone formation on both cortical and cancellous bone surfaces and appears to be well tolerated when administered for up to

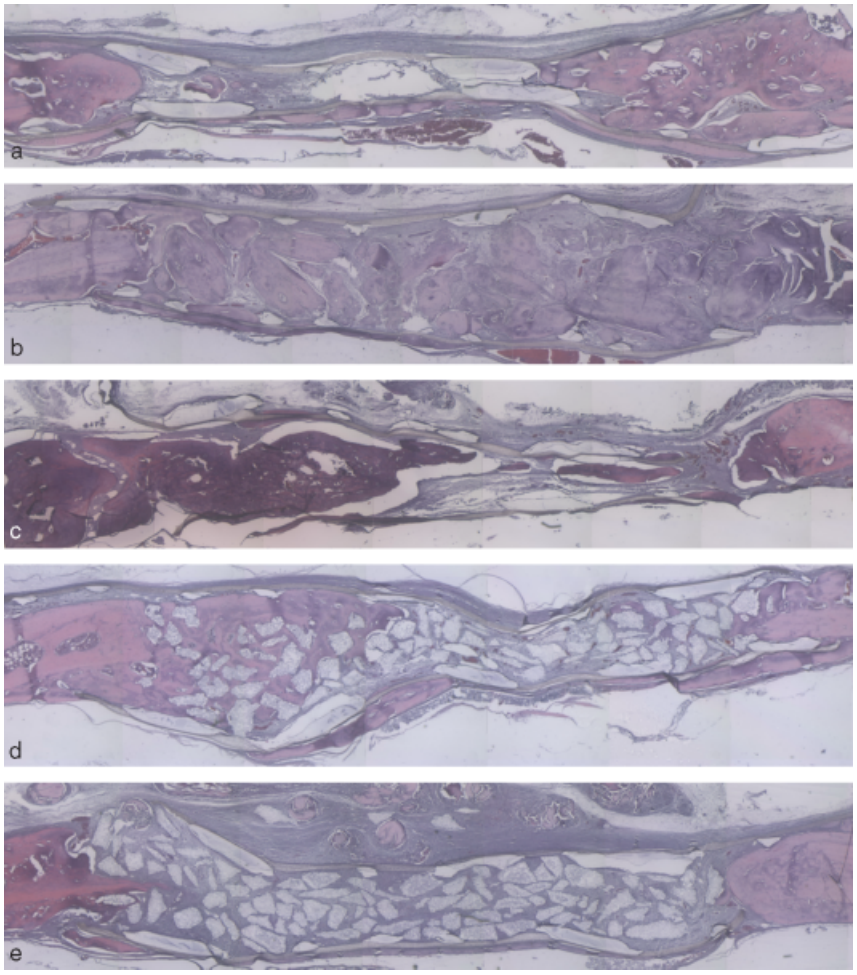

Fig. 5. Representative photomicrographs showing (a) sham-surgery control, (b) demineralized freeze-dried bone, (c) polytetrafluoroethylene (PTH), (d) β-tricalcium phosphate (β-TCP), and (e) PTH/β-TCP specimens following an 8-week healing interval. All but the PTH/β-TCP specimen show new bone formation. The β-TCP biomaterial occupies a large area of the defect site in the β-TCP and PTH/β-TCP specimens (haematoxylin & eosin).

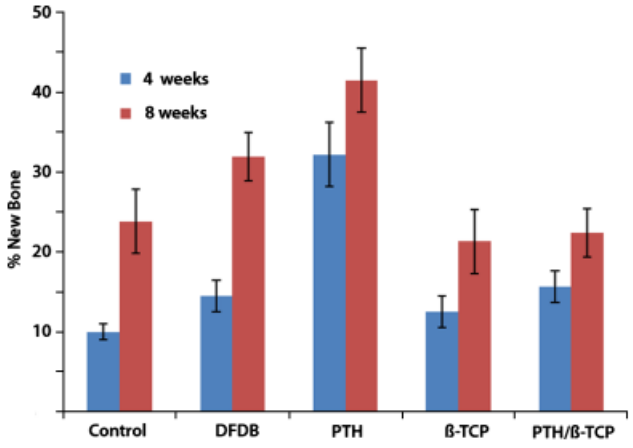

Fig. 6. Histometric new bone formation (means $\pm$ SEM in %). At 4 weeks, the polytetrafluoroethylene (PTH) group exhibited significantly greater new bone formation compared with the demineralized freeze-dried bone (DFDB), β-tricalcium phosphate (β-TCP), PTH/β-TCP, and sham-surgery control groups ($p<0.014$). At 8 weeks, the PTH group exhibited significantly greater new bone formation than that at β-TCP, PTH/β-TCP, and sham-surgery control sites ($p<0.025$). The DFDB group showed increased bone formation from 4 to 8 weeks ($p<0.006$).

one year. PTH administered s.c. is likely to have systemic effects on skeletal tissues, although this was not investigated within the limitations of this study. Locally addressing craniofacial defects may be preferable to systemic exposure and as a result further research into possibly involving intermittent time-limited local administration of PTH for craniofacial (Jung et al. 2007) but also defects elsewhere in the axial and appendicular skeleton appears warranted. If successful, this may reveal considerably different dosing schemes than that of systemic application as in cases of osteoporosis.

The multi-porous granular (250–500 µm) β-TCP biomaterial in the present study should be considered an osteoconductive ceramic; that is a matrix that would enhance osteogenic bone formation (Zijderveld et al. 2005, Horch et al. 2006). The results of this study poorly support this notion. Similar amounts of bone formation or bone fill were observed at the sham-surgery control and at sites implanted with β-TCP at 4 and at 8 weeks suggesting that this biomaterial is not readily or remarkably osteoconductive. In fact, similar amounts of bone formation or bone fill were also observed in animals receiving the PTH/β-TCP combination at 4 and at 8 weeks, significantly lower than that in sites exposed to PTH alone suggesting that the β-TCP biomaterial actually may obstruct bone formation. Nevertheless, slowly resorbing or non-resorbing bone biomaterials such as the β-TCP product herein still maintain everyday clinical use. Considering that the bone metabolic rate and likely bioresorption of implanted biomaterials is greater in rodent models than in humans, the observations herein may have considerable clinical implications given the β-TCP biomaterial vastly remained intact at 8 weeks post-implantation in turn suggesting that an extended tissue maturation period must pass before such sites may be used for placement of endosseous oral implants.

DFDB served as a benchmark control. The DFDB biomaterial supported greater bone formation at 8 weeks compared with both β-TCP treatments and sham-surgery; statistically significant increases from 4 to 8 weeks indicative of pronounced late bone formation. The histometric results support previous studies elucidating DFDB's osteoconductive and possible osteoinductive properties in rodent models (Lim et al. 2000). Notably human DFDB was used.

Although rat DFDB was considered, human DFDB has been shown to be effective in this defect model, closing most of the osteotomy defect when used with a barrier device (Lim et al. (2000). The use of human DFDB also allowed immediate comparisons with previous studies. Delayed bone formation shown in this study may demonstrate the true osteoconductive/osteoinductive potential of this biomaterial in a rat model, but bone formation may also have been affected (delayed) due to circumstances created by the barrier devices obstructing critical vascular and cellular elements such as has been shown for rhBMP-2 constructs (Zellin & Linde 1997).

Comparing the radiographic and histometric analysis, it becomes readily evident that the radiographic surrogate estimates of local bone formation do not necessarily correlate well with the histologic recordings in particular for sites receiving radiopaque biomaterials. Thus the results of the radiographic analysis received limited attention in the evaluation of local bone formation for sites also implanted with the β-TCP biomaterial. These observations corroborate with earlier reports designed at comparing radiographic and histometric analysis of bone fill in rat calvarial osteotomy defects concluding that ''low accuracy was observed when radiographic evaluations were used in identifying and characterizing bone fill in the rat calvaria osteotomy defects. Assessment of bone healing in animal models aiming at treatment recommendations for clinical application must not solely be based on radiographic analysis, but should be confirmed using histologic observations'' (Pryor et al. 2006). The observations in this study indeed underscore this necessity.

In conclusion, systemic administration of PTH significantly stimulates local bone formation. Bone formation was significantly limited by the β-TCP biomaterial.

Acknowledgements

The authors thank Mr. Eugene Cauley, SPC Robin McEntee, and MAJ Stevens-DeValle for project preparation, Mr. Royce Runner for research expertise and Mrs. Jordan Mastrodonato for medical illustrations for the first place Billy Pennel Research Competition, 2007 Southern Academy of Periodontology Summer Meeting. The authors thank the American Academy of Periodontology for recognizing this research as a basic science category finalist in the 2008 Balint Orban Memorial Competition. In addition, the authors thank the US Army Dental and Trauma Research Detachment for awarding this research the first place in the 2008 Major General Joseph L. Bernier Research Award Competition, Category I. The opinions expressed in this article do not represent the views of the US Department of Defense, the Department of the Army, or the US Army Dental Corps. Use of any commercial products in this project does not imply endorsement by the US Government. Funding was provided in its entirety from the USA DENTAC.

References

Ahn, S. H., Kim, C. S., Suk, H. J., Lee, Y. J., Choi, S. H., Chai, J. K., Kim, C. K., Han, S. B. & Cho, K. S. (2003) Effect of recombinant human bone morphogenetic protein-4 with carriers in rat calvarial defects. *Journal of Periodontology* **74**, 787–797.

Alkhiary, Y. M., Gerstenfeld, L. C., Krall, E., Westmore, M., Sato, M., Mitlak, B. H. & Einhorn, T. A. (2005) Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1-34). *The Journal of Bone and Joint Surgery* **87A**, 731–741.

Andreassen, T. T. & Cacciafesta, V. (2004) Intermittent parathyroid hormone treatment enhances guided bone regeneration in rat calvarial bone defects. *Journal of Craniofacial Surgery* **15**, 424–429.

Cosman, F. (2005) Anabolic therapy for osteoporosis: parathyroid hormone. *Current Osteoporosis Reports* **3**, 143–149.

Deal, C. & Gideon, J. (2003) Recombinant human PTH 1-34 (Forteo): an anabolic drug for osteoporosis. *Cleveland Clinic Journal of Medicine* **70**, 585–601.

Horch, H. H., Sader, R., Pautke, C., Neff, A., Deppe, H. & Kolk, A. (2006) Synthetic, pure phase beta-tricalcium phosphate ceramic granules (Cerasorb) for bone regeneration in the reconstructive surgery of the jaws. *International Journal of Oral and Maxillofacial Surgery* **35**, 708–713.

Isaksson, S. & Alberius, P. (1992) Comparison of regenerative capacity elicited by demineralized matrix of different embryonic origins. *Journal of Cranio-Maxillo-Facial Surgery* **20**, 73–80.

Jilka, R. L., Weinstein, R. S., Bellido, T., Roberson, P., Parfitt, A. M. & Manolagas, S. C. (1999) Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. *The Journal of Clinical Investigation* **104**, 439–446.

Jung, R. E., Cochran, D. L., Domken, O., Seibl, R., Jones, A. A., Buser, D. & Hämmerle, C. H. (2007) The effect of matrix bound parathyroid hormone on bone regeneration. *Clinical Oral Implants Research* **18**, 319–325.

Kuemmerle, J. F. (2005) Endogenous IGF-1 protects human intestinal smooth muscle cells from apoptosis by regulation of GSK-3β activity. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **288**, G101–G110.

Lim, S. C., Lee, M. J. & Yeo, H. H. (2000) Effects of various implant materials on regeneration of calvarial defects in rat. *Pathology International* **50**, 594–602.

Madore, G. R., Sherman, P. J. & Lane, J. M. (2004) Parathyroid hormone. *The Journal of the American Academy of Orthopaedic Surgeons* **12**, 67–71.

Marx, J. (2004) Coming to grips with bone loss. *Science* **305**, 1420–1422.

Matzenbacher, S. A., Mailhot, J. M., McPherson, J. C. III, Cuenin, M. F., Hokett, S. D., Sharawy, M. & Peacock, M. E. (2003) In vivo effectiveness of a glycerol-compounded demineralized freeze-dried bone xenograft in the rat calvarium. *Journal of Periodontology* **74**, 1641–1646.

Pang, E. K., Im, S. U., Kim, C. S., Choi, S. H., Chai, J. K., Kim, C. K., Han, S. B. & Cho, K. S. (2004) Effect of recombinant human bone morphogenetic protein-4 dose on bone formation in a rat calvarial defect model. *Journal of Periodontology* **75**, 1364–1370.

Pöhling, S., Pippig, S. D., Hellerbrand, K., Siedler, M., Schütz, A. & Dony, C. (2006) Superior effect of MD05, beta-tricalcium phosphate coated with recombinant human growth/differentiation factor-5, compared to conventional bone substitutes in the rat calvarial defect model. *Journal of Periodontology* **77**, 1582–1590.

Precheur, H. (2007) Bone graft materials. *Dental Clinics of North America* **51**, 729–746.

Prolo, D. J., Pedrotti, P. W., Burres, K. P. & Oklund, S. (1982) Superior osteogenesis in transplanted allogenic canine skull following chemical sterilization. *Clinical Orthopaedics and Related Research* **168**, 230–242.

Pryor, M. E., Susin, C. & Wikesjö, U. M. E. (2006) Validity of radiographic evaluations of bone formation in a rat calvaria osteotomy defect model. *Journal of Clinical Periodontology* **33**, 455–460.

Quattrochi, E. & Kourlas, H. (2004) Teriparatide: a review. *Clinical Therapeutics* **26**, 841–854.

Schmitz, J. P. & Hollinger, J. O. (1986) The critical size defect as an experimental model for craniomandibular nonunions. *Clinical Orthopaedics and Related Research* **205**, 299–308.

Schmitz, J. P., Schwartz, Z., Hollinger, J. O. & Boyan, B. D. (1990) Characterization of rat calvarial nonunion defects. *Acta Anatomica* **138**, 185–192.

Shiratori, K., Matsuzaka, K., Koike, Y., Murakami, S., Shimono, M. & Inoue, T. (2005) Bone formation in ß-tricalcium phosphate-filled bone defects of the rat femur: morphometric analysis and expression of bone related protein mRNA. *Biomedical Research* **26**, 51–59.

Skripitz, R., Andreasson, T. T. & Aspenberg, P. (2000) Parathyroid hormone (1–34) increases

the density of rat cancellous bone in a bone chamber. *The Journal of Bone and Joint Surgery* **82-B**, 138–141.

Skripitz, R. & Aspenberg, P. (2004) Parathyroid hormone-a drug for orthopedic surgery? *Acta Orthopaedica Scandinavica* **75**, 654–662.

Zellin, G. & Linde, A. (1997) Importance of delivery systems for growth-stimulatory factors in combination with osteopromotive membranes. An experimental study using rhBMP-2 in rat mandibular defects. *Journal of Biomedical Materials Research* **35**, 181–190.

Zijderveld, S. A., Zerbo, I. R., van den Bergh, J. P., Schulten, E. A. & Bruggenkate, C. M. (2005) Maxillary sinus floor augmentation using a β-tricalcium phosphate (Cerasorb) alone to autogenous bone grafts. *The International Journal of Oral & Maxillofacial Implants* **20**, 432–440.

Address:
Jonathan I. Yun
851 Ted A Crozier Sr. Blvd Apt#711
Clarksville, TN 37043
USA
E-mail: jonathan.yun@us.army.mil

Clinical Relevance

Scientific rationale for the study: Systemic administration of PTH (1–34) supports osteogenic bone formation. Osteoconductive matrices are commonly used in support of alveolar augmentation. The objective of this study was to evaluate a potential synergy between PTH enhanced osteogenesis and a surgically implanted synthetic β-TCP bone biomaterial serving as a matrix to support new bone formation.

Principal findings: Systemic administration of PTH effectively stimulates local bone formation. Bone formation was significantly limited by the β-TCP biomaterial.

Practical implications: Locally addressing craniofacial defects may be preferable to systemic approaches thus development of intermittent time-limited local administration of PTH appears to be a desirable venue to support alveolar augmentation.