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Inflammatory mediator release following bone grafting in humans: a pilot study

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Abstract

Aim: The aim of this pilot study was to track markers of periodontal inflammation and bone resorption associated with decalcified freeze-dried bone allografts.

Material and Methods: Eleven subjects completed standardized treatment of intrabony defects $\geqslant 3$ mm with allografts. Gingival crevicular fluid was collected from the defect site and an adjacent interproximal site within the surgical field at baseline, 2, 4, and 8 weeks post-operatively, and analysed for biochemical markers of inflammation/bone resorption. Probing depth, recession, bleeding on probing, plaque, and 6-month radiographic bone height change were measured.

Results: Both prostaglandin E_2 ($p = 0.007$) and bone-specific type 1 collagen ($p = 0.01$) increased in crevicular fluid after 2 weeks in the bone graft sites. Matrix metalloproteinase-9 levels remained constant over time. There were positive correlations between prostaglandin levels during the first 8 weeks and bone height change over 6 months.

Conclusions: Periodontal bone grafts stimulate an inflammatory response during the first 2 weeks post-operatively, and the potential negative effects of inhibiting prostaglandins post-operatively should be investigated further.

Key words: bone resorption; inflammation; matrix metalloproteinase; periodontal allografts; prostaglandin E_2

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Bone replacement grafts remain among the most widely used therapeutic strategies for the correction of periodontal osseous defects (Wang et al. 2005). A commonly used graft material is a demineralized freeze-dried bone allograft (DFDBA). Observational and controlled studies generally document improvements in clinical parameters following placement of graft materials (Brunsvold & Mellonig 1993, Laurell et al. 1998). Mean gains in clinical attachment level from 1.9 to 2.9 mm and recession of 0.2 mm have been reported (Quintero et al. 1982, Mellonig 1984).

In the periodontal literature, it has long been established that plaque-associated inflammation adversely effects wound healing. In a study of post-operative plaque control, it was demonstrated that patients who received frequent recalls for oral hygiene during early healing had fewer sites with clinical attachment loss, more sites with attachment gain, and fewer periodontal pockets (Westfelt et al. 1983). In the presence of inflammation, break-down of the fibrin clot in the healing gingival wound is accelerated, resulting in epithelial migration into the wound and apical migration of the epithelial attachment to the teeth (Yumet & Polson 1985). The authors of this work suggested that, in the presence of plaque-associated inflammation, neutrophils produce gelatinase (matrix metalloproteinase-9, MMP-9), which readily breaks down the immature collagen of a healing wound, resulting in apical migration of epithelium. Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) and prostaglandin E_2 (PGE_2) are biochemical markers of bone collagen degradation and inflammation, respectively, and have been associated with bone resorption and bone loss (Eriksen et al. 1993, Preshaw et al. 1999). To ensure better surgical outcomes, it is thought that extraneous inflammation from plaque and other sources should be minimized. In addition, all the above mediators

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

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can be reduced by systemic drug therapy [i.e., MMPs by tetracyclines, ICTP by bisphosphonates, and PGE_2 by non-steroidal anti-inflammatory drugs (NSAIDs)]. We hypothesized in this study that periodontal bone grafts with DFDBA would cause increases in some of these mediators of inflammation/bone degradation above baseline. Understanding the profile of these markers during early wound healing following a bone regenerative procedure may suggest how they are associated with bone growth and how pharmacological interventions may alter clinical outcomes.

We also hypothesized that increased levels of PGE_2, ICTP, or MMP-9 [sampled in gingival crevicular fluid (GCF)] during the first 8 weeks of wound healing would be associated with less radiographic bone growth at 6 months after grafting with DFDBA.

Material and Methods

Patient selection

This research was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki. Twelve subjects were enrolled for this study at the University of Nebraska Medical Center College of Dentistry from April 2004 to June 2005 and signed a UNMC Institutional Review Board-approved consent form. Subjects ranged in age from 21 to 86 years (mean ± standard error = 61 ± 5 years).

Inclusion criteria

The inclusion criteria included: (1) anticipated a quadrant of periodontal surgery with interproximal grafts for two- to three-walled intrabony defects ⩾3 mm deep, not adjacent to a furcation; (2) had an additional interproximal site to monitor the effects of surgical bone exposure alone in the same surgical quadrant that would be included in the mucogingival flap reflection, but not receive a bone graft (sites not necessarily appropriate for bone grafts); and (3) in good general health appropriate for periodontal flap surgery as determined by their periodontal care provider, including no conditions at risk for excessive bleeding or transient bacteremia sequelae as a result of periodontal surgery or post-operative probing. No standardized pre-surgical oral hygiene programme was carried out.

Table 1. Data collection schedule and sequence

Baseline	Week 2	Week 4	Week 8	Month 6
Standardized Bitewing Radiograph				Standardized Bitewing Radiograph
Plaque detection	Plaque detection	Plaque detection	Plaque detection	Plaque detection
GCF sampling	GCF sampling	GCF sampling	GCF sampling	
Recession				Recession
Probing depth				Probing depth
Bleeding on probing				Bleeding on probing

GCF, gingival crevicular fluid.

Subjects who were pregnant, allergic to local anaesthetics or narcotics, or taking drugs known to affect bone metabolism such as oestrogen, bisphosphonates, tetracyclines, and steroidal and non-steroidal anti-inflammatory drugs were excluded. Calcium and vitamin D were allowed. One subject reported being a current smoker.

Study design

Subjects were examined at baseline (just before treatment) and 2, 4, 8 weeks, and 6 months post-operatively according to the sequence in Table 1. Following baseline sampling, a full-thickness mucoperiosteal flap was elevated to expose both the graft and bone exposure sites. The graft site was debrided, the root surfaces were prepared with root planing, and the defect was grafted with DFDBA (LifeNet, Virginia Beach, VA, USA). The same DFDBA lot was used for all subjects. The post-operative regimen included oral hygiene instructions and twice-daily (0.12%) chlorhexidine digluconate rinses. For post-operative discomfort, subjects were prescribed acetaminophen or an acetaminophen/hydrocodone combination.

Clinical measurements

Bone exposure and graft sites were evaluated for the presence of supragingival plaque at each clinical visit. Plaque was recorded as present if it could be observed when running a periodontal probe across the surface of the tooth at the level of the gingival margin.

Probing depths and recession from the cementoenamel junction were measured pre-operatively (baseline) and at 6 months post-operatively with the UNC 15 Probe and recorded to the nearest millimetre. Clinical attachment levels (CAL) were calculated as the sum of these values. Bleeding on probing (BOP) was measured 30 s after probing.

GCF sampling

GCF samples were collected from the sulcus adjacent to the graft site and the bone exposure site. Before sample collection, plaque was removed from the supragingival tooth surface with a curette, the area was isolated with gauze, and dried by light application of compressed air. Sample collection involved placement of a methylcellulose strip (Periopaper, ProFlow Inc., Amityville, NY, USA) into the sulcus until slight resistance was met and leaving it in place for 30 s. Samples were then placed in a sterile vial, frozen immediately at −70°C, and stored until analysis.

Analysis of biochemical markers

All samples were thawed and brought to room temperature. The GCF samples (on methylcellulose strips) were eluted into 200 μl PBS by placing on a shaker for 90 min. All samples were placed on a vortex device before analysis.

Total protein

The amount of protein in GCF samples was quantified using a kit (BCA™ Protein Assay Kit, Pierce Chemical, Rockford, IL, USA) based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. Briefly, a volume of 10 μl of each unknown sample and standard was mixed with 200 μl of a working reagent at 37°C for 30 min. After cooling the plate to room temperature, the absorbance was measured at 562 nm. The minimum detection limit was 25 μg/ml.

PGE_2

An immunoassay (ACE™ Competitive Enzyme Immunoassay, Cayman Chemical, Ann Arbor, MI, USA) was used to quantify the amount of PGE_2 in samples, based on the competition between

PGE_2 and a PGE_2-acetylcholinesterase (AChE) conjugate (PGE_2 tracer) for a limited amount of PGE_2 monoclonal antibody. In brief, a volume of 50 μl of each sample was placed by a pipette into a microtitre well. Then, 50 μl of PGE_2 AChE tracer and PGE_2 monoclonal antibody were added to each well. The plates were then covered and incubated for 18 h at 4°C, washed and 200 μl of Ellman's reagent were added to each well, the plate was covered, and then placed on an orbital shaker and allowed to develop for 90 min. The absorbance was measured at 405 nm on a plate reader. The minimum detection limit for this kit was 15 pg/ml.

ICTP

An immunoassay kit (UniQ Enzyme Immunoassay Kit, Orion Diagnostica, Espoo, Finland) was used for quantification of ICTP in samples, based on the competitive immunoassay technique. Briefly, 50 μl of calibrator, control, and sample were pipetted into appropriate microtitre wells, and then 50 μl of an ICTP enzyme conjugate and ICTP antiserum were pipetted into all wells except blanks. The antiserum was applied to all wells within 3 min. Wells were incubated on a plate shaker for 2 h and then washed four times with the wash solution. 100 μl of an ICTP substrate were added to the wells, and plates were incubated on a plate shaker for 30 min. The enzyme reaction was stopped by adding 100 μl of a stopping solution to all wells and shaking for 15–30 s. Samples were then read on a photometric plate reader at an absorbance of 450 nm. The minimum detection limit was 300 pg/ml.

MMP-9

Zymography (Novex Zymogram, Invitrogen Experimental Technology, San Diego, CA, USA) was used to quantitate MMP-9 activity from 10 μg samples as described previously (Reinhardt et al. 2005). These pre-cast gels consisted of a 10% sodium dodecyl sulphate (SDS)-polyacrylamide gel with 0.1% of gelatin incorporated as a substrate. The gels were run under non-reducing conditions with 1 × Tris-glycine SDS running buffer at 125 V. The gels were then renatured with 2.5% Triton X-100 for 30 min., rinsed briefly, and developed with 50 mmol/l Tris/HCL buffer, pH 7.6, containing 10 mmol/l $CaCl_2$ at 37°C overnight. Proteinases were easily identified as clear bands against a dark Coomassie blue-stained background. The zymograms were then densitometrically scanned using a digital program (Eastman Kodak, Rochester, NY, USA). MMP-9 was quantified in arbitrary units based on band densities from the zymograms.

Standardized radiographs

A single vertical bitewing radiograph was taken of the graft and bone exposure sites at baseline and 6 months with positioning standardized using a cephalometric head positioner according to Jeffcoat et al. (1987). The standardized radiograph was then digitized with a digital scanner (Polaroid Sprintscan 35, Waltham, MA, USA) and analysed with computer software (ElectroMedical Systems, Richardson, TX, USA) for depth of the intrabony defect from a fixed reference point to where the periodontal ligament space was uniform in width. All measurements were conducted by two evaluators who had no knowledge of subject or appointment information. The change in defect bone level (ΔBL_{6mos}) was calculated by subtracting the measurement of the baseline radiograph from the measurement of the 6-month follow-up radiograph. Discrepancies in change between evaluators of $\geqslant 1$ mm were identified and measurements were repeated by both examiners. The mean values for ΔBL_{6mos} were then calculated for use in the statistical analysis.

Statistical analysis

Biochemical marker levels at bone exposure and graft sites among each of the four sampling periods (baseline, 2, 4, and 8 weeks, post-operatively) were compared using analysis of variance. For significant time factors, means were separated with a protected LSD test. Mean data were expressed with standard error of the mean.

The amount of PGE_2, ICTP, and MMP-9 in each 30-s GCF sample was calculated by adjusting for elution volume and volume analysed, and reported as total amount per 30 s sample. For secondary analysis comparing biochemical markers with defect bone fill, amounts of biochemical markers in GCF were also normalized by dividing the concentrations of biochemical markers by the concentrations of total protein. Evaluation of clinical parameters and biochemical markers during the first 8 weeks was compared with defect bone fill at 6 months using Pearson and Spearman correlation coefficients.

Results

Eleven (eight males, three females) of the 12 subjects enrolled attended all examination visits, and provided a full set of data for analysis. There were no reported post-operative infections or unexpected adverse events, except for one subject, who exfoliated the graft material before the week 2 post-operative visit.

Clinical parameters

The clinical measurements are summarized in Table 2. Six months after flap surgery, some improvement in BOP was noted in the graft group, but the plaque levels (73%) and BOP (45%) remained high. The CAL and probing depth remained constant in the bone exposure group 6 months post-surgery, while the mean CAL improved 1.5 mm and PD decreased 2.1 mm at graft sites.

Biochemical markers at bone exposure sites and graft sites across time

The PGE_2 levels in the bone graft group increased after 2 weeks relative to baseline ($p = 0.007$), and then returned to

Table 2. Clinical measurements

	Site	Baseline	6 months
Plaque (%)	Bone exposure	64	55
	Graft	82	73
Bleeding on probing (%)	Bone exposure	18	18
	Graft	64	45
Clinical attachment level (mm ± SEM)	Bone exposure	4.8 ± 0.8	4.9 ± 0.7
	Graft	9.9 ± 1.0	8.4 ± 0.7
Probing depth (mm ± SEM)	Bone exposure	3.2 ± 0.4	3.2 ± 0.4
	Graft	7.8 ± 0.9	5.7 ± 0.7

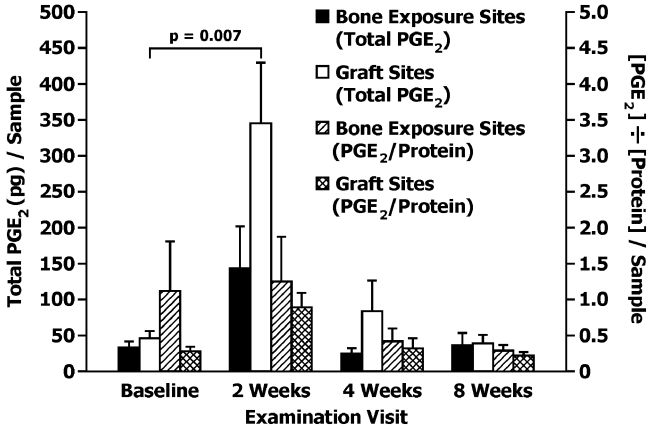

Fig. 1. Total prostaglandin E_2 (PGE_2) and PGE_2/protein in gingival crevicular fluid (GCF) for bone exposure and graft sites (mean ± standard error) during early healing. The bracket indicates difference between baseline and 2 weeks in the graft group.

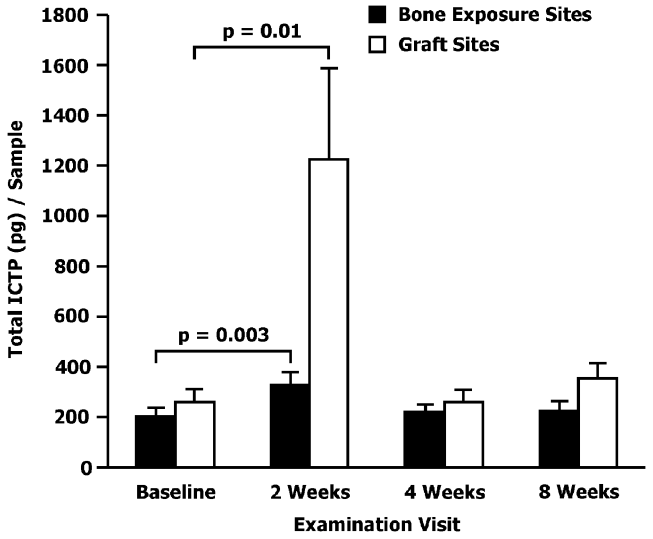

Fig. 2. Total ICTP in gingival crevicular fluid (GCF) for bone exposure and graft sites (mean ± standard error) during early healing. Brackets indicate differences between baseline and 2 weeks in both groups.

near baseline at 4 and 8 weeks (Fig. 1). A similar pattern was observed for PGE_2/protein for graft sites. Bone exposure sites did not show any significant changes across time for PGE_2. For total ICTP in GCF, both graft and bone exposure values were the highest two weeks post-surgery relative to baseline ($p \leqslant 0.01$, Fig. 2). For total MMP-9 in GCF, the levels in both groups did not vary across time (Fig. 3).

Radiographic parameters

The mean change in defect bone level at 6 months (mean ΔBL_{6mos}) was 1.2 ± 0.8 mm. Five sites showed <1 mm gain in bone level (including three sites which lost bone), while six sites gained an average of 2.9 ± 0.7 mm of defect bone fill. Only one treatment site showed bone loss of >0.5 mm. The mean bone-level change at bone exposure sites from baseline to 6 months was minimal (-0.1 ± 0.3 mm).

Correlations between clinical/biochemical markers and changes in defect bone level

Comparisons of clinical measures (probing depth, CAL, and BOP at the baseline examination and plaque presence at each examination) with ΔBL_{6mos} showed that none of these clinical parameters were significantly correlated with defect bone fill (data not shown).

Comparisons of biochemical marker levels with ΔBL_{6mos} at graft sites showed two statistically significant positive correlations (Pearson): (1) between baseline levels of PGE_2 in 30 s GCF samples (total PGE_2) and ΔBL_{6mos} ($r = 0.67$, $p = 0.02$; Fig. 4) and (2) between PGE_2/protein in GCF at 8 weeks and ΔBL_{6mos} ($r = 0.65$, $p = 0.03$; Fig. 5). ICTP and MMP-9 values did not show any significant relationship with ΔBL_{6mos} (data not shown). However, GCF MMP-9 showed a weak trend towards a negative correlation with ΔBL_{6mos} at 8 weeks ($r = -0.44$, $p = 0.18$). Results similar to Pearson correlations were seen when Spearman correlation coefficients were calculated for the study data.

Discussion

Reports tracking the release of PGE_2, ICTP, and MMP-9 following periodontal regeneration procedures in humans are rare. Inflammatory mediators in GCF come from a mixture of sources, including the healing bone surface and marginal gingival inflammation. However, GCF sampling has the advantage of being minimally disruptive to the healing process and represents the standard for following inflammatory mediators during periodontal wound healing. Some existing reports of flap surgery measured GCF mediators first at 3 months post-surgery (Alexander et al. 1996, Gapski et al. 2004) or with very few subjects for statistical analysis (Engstrom et al. 2001). Sarment et al. (2006) measured ICTP in periodontal wound fluid (WF, essentially GCF collected for a constant length of time 10 s at the test site in the healing flap wound), including earlier post-surgical time points (3 and 6 weeks). They found that periodontal defects treated with β-tricalcium phosphate (β-TCP) along with platelet-derived growth factor (PDGF) did not elevate ICTP significantly above baseline at 3 or 6 weeks. A similar protocol with β-TCP+PDGF led to increases in WF ICTP after 3–5 days (Cooke et al. 2006). These data are in agreement with the data from the current study showing GCF ICTP to be significantly higher after 2 weeks, but near baseline after 4 weeks (Fig. 2), suggesting that ICTP activity following grafting procedures occurs primarily between 3 and 14 days. Flap exposure alone, as defined by bone exposure sites

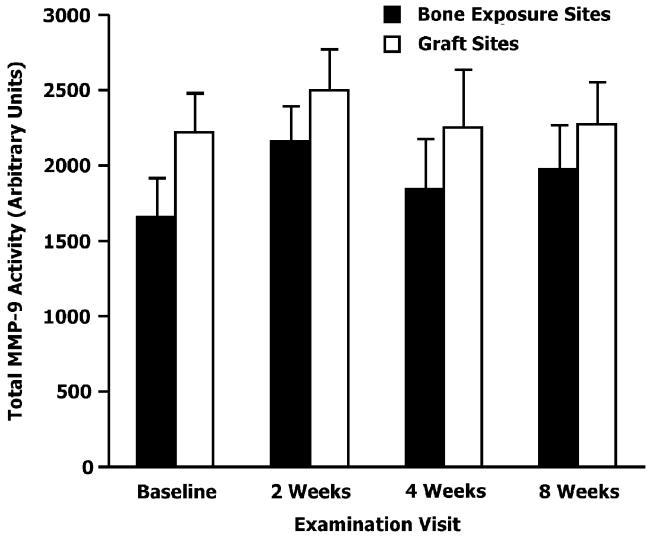

Fig. 3. Total MMP-9 activity in gingival crevicular fluid (GCF) for bone exposure and graft sites (mean ± standard error) during early healing. No significant differences were found among time periods.

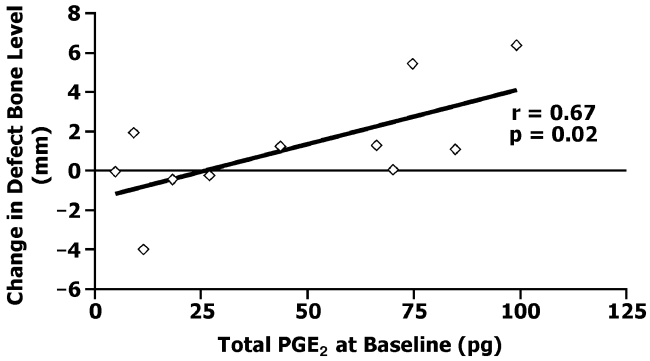

Fig. 4. Scatter plot of total prostaglandin E_2 (PGE_2) in gingival crevicular fluid (GCF) from graft sites at baseline *versus* bone-level change at 6 months.

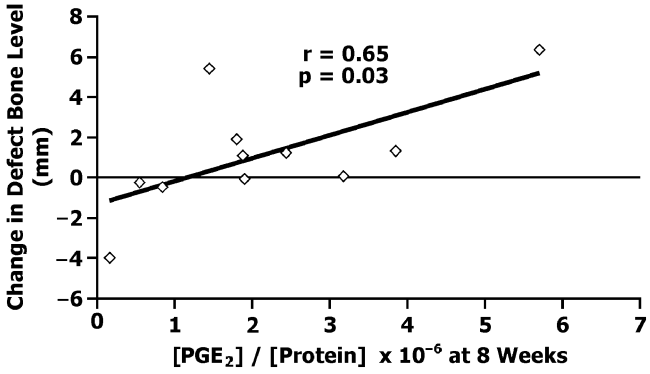

Fig. 5. Scatter plot of concentrations of prostaglandin E_2 (PGE_2)/protein in gingival crevicular fluid (GCF) from graft sites at 8 weeks *versus* bone-level change at 6 months.

in the current study, also resulted in elevated GCF ICTP at 2 weeks (Fig. 2). GCF was collected over a constant time period (30 s) and the total amounts of mediator were used rather than concentrations. Concentrations have potential for overestimations resulting from division by small volumes of GCF, such as in less inflamed sites (Lamster 1997). GCF ICTP returned to baseline levels after 4 and 8 weeks. Gapski et al. (2004) showed an increase in GCF ICTP after 12 weeks, although it was not reported as being significantly different from baseline.

The current report helps to define the GCF PGE_2 and MMP-9 patterns after bone grafting. GCF PGE_2 (Fig. 1) followed a pattern similar to ICTP, while MMP-9 remained unchanged relative to baseline (Fig. 3). Engstrom et al. (2001) reported a numerical increase (no statistical analyses) in PGE_2 concentration 2, 4, and 12 weeks after surgical implantation of hyaluronan and a bioabsorbable membrane on intrabony pockets, but no increase with membrane alone. In addition, a variety of periodontal surgeries (flap debridement, resective, or regenerative procedures) have been reported to lead to no increase in PGE_2 concentrations after 3 months relative to baseline (Alexander et al. 1996). No reports of MMP-9 levels following human periodontal bone-regenerative procedures could be found in the literature.

The baseline values for total PGE_2 were essentially identical in bone exposure and graft sites in spite of the higher supragingival plaque, BOP (clinical inflammation), and probing depths in the graft group. While GCF PGE_2 levels have been reported to be significantly correlated to gingival inflammatory indices (Nakashima et al. 1994), other reports found no highly positive relationship between GCF PGE_2 and pocket depth (PD) or BOP (Offenbacher et al. 1993, Ohshima et al. 2002). Alexander et al. (1996) showed that BOP reductions during periodontal therapy were not associated with concurrent reductions in total GCF PGE_2, and Preshaw et al. (1999) demonstrated that GCF PGE_2 levels could change without alteration in BOP. Taken together, these papers are consistent with our findings that elevated BOP and PD in graft sites compared with bone exposure sites were not associated with significantly elevated PGE_2. Even so, PGE_2 was significantly elevated after 2 weeks only in the grafted sites. ICTP levels were elevated at both graft and bone exposure sites 2 weeks post-operatively, potentially from bone resorption following surgical bone exposure. GCF MMP-9 was not elevated in either group at any post-operative time point (Fig. 3). Therefore, the three biochemical mediators measured in this study reacted to flap surgery with different patterns. The ideal control of a matched two- to three-walled intrabony defect $\geqslant 3$ mm deep,

surgically exposed but not grafted, was not practical in this human study for obvious logistical and ethical reasons.

Radiographic defect bone fill as evaluated in this study is representative of the clinical restoration of bone tissue in a periodontal defect. Changes in radiographic measurements after surgical treatments of interproximal vertical defects have been shown to yield almost identical results as CAL or probing to bone (Zybutz et al. 2000). Comparative studies of pre-treatment bone levels and post-therapy defect bone fill with 12-month re-entry bone measurements showed that linear radiographic analysis significantly underestimates pre-treatment bone loss and post-treatment defect bone fill (Tonetti et al. 1993, Zybutz et al. 2000), therefore indicating that the benefits of treatment in the current study may have been underestimated by the radiographic measured outcomes.

The mean change in defect bone level at 6 months was 1.2 ± 0.8 mm. A recent systematic review reported a mean increase in defect fill of 0.8 mm using DFDBA in intrabony defects (Reynolds et al. 2003). However, the mean result in defect bone fill does not account for individual variability and tends to obscure the clinical significance of the treatment. Five of the 11 sites showed <0.5 mm defect fill and the other six showed >1 mm bone fill, averaging 2.9 mm. Dividing subjects into these two groups shows a relatively even distribution of those who showed clinically unfavourable and favourable outcomes, respectively. Plaque control in the study subjects was less than ideal. Plaque was identified at graft sites in 80% of the evaluation visits. Patients were not subjected to a standardized pre-operative oral hygiene programme or post-operative regimen other than customized instruction and twice-daily chlorhexidine rinses for about 1 week. The absence of an aggressive plaque control programme and the more complex supragingival anatomy of the graft sites probably led to the relatively high BOP levels after 6 months. It should be noted that bone exposure sites in the same vicinity maintained a low 18% bleeding index throughout the study. Because poor plaque control has been associated with poor periodontal wound-healing outcomes (Yumet & Polson 1985), the incidence of plaque in this study may have limited bone fill. However, there was no significant correlation between plaque or clinical inflammation (BOP) and change in linear bone height. Similar findings have been reported with outcomes for bone grafts in Class II furcation defects (Bowers et al. 2003, Tsao et al. 2006). In addition, the ubiquitous presence of plaque may further explain why no relationship was identified between the presence of plaque and bone graft success.

In contrast to the lack of impact regarding clinical signs of inflammation on bone fill, defect bone fill at 6 months was positively correlated with PGE_2 in GCF graft sites at baseline (Fig. 4) and PGE_2/protein in GCF at 8 weeks post-operatively (Fig. 5). A similar effect was observed in an irrigant of bone near the graft site after 4 weeks compared with 6-month bone fill ($r = 0.81$, $p = 0.003$; data not shown), and linear regression analysis suggested that PGE_2/protein levels were predictive of BL_{6mos} ($p = 0.001$). These findings are in contrast with the expected results because it was originally believed that elevated markers of inflammation would result in decreased defect bone fill. GCF PGE_2 was usually elevated in sites that had better bone fill results post-grafting (Figs 4 and 5). Regarding pre-operative PGE_2 levels in intrabony sites (Fig. 4), high levels may reflect inflammation associated with the periodontitis lesion. However, the mean PGE_2 values were not different between the graft sites and bone exposure sites that had less PD and clinical inflammation (Fig. 1 and Table 2). This suggests that GCF PGE_2-associated inflammation may be an indicator of bone graft success, or that PGE_2 is potentially involved in stimulating bone growth during healing.

PGE_2 is considered to be a potent mediator of bone resorption because of its ability to stimulate osteoclast differentiation (Liu et al. 2005). However, other studies have suggested an unexpected bone–sparing characteristic of PGE_2. Li et al. (1995) showed that PGE_2 administration prevented bone loss induced by orchidectomy in rats. PGE_2 has also been shown to directly inhibit the bone-resorbing activity of functionally mature osteoclasts (Mano et al. 2000). In addition to bone-sparing properties, PGE_2 appears to play a key role in bone growth. Cyclooxygenase (COX) inhibitors are commonly used to block the production of PGE_2. The combined use of simvastatin gel, a bone-growth stimulant, with a COX-2 inhibitor (NS-398) or a general COX-inhibitor (indomethacin), yielded decreased bone growth in the rat mandible (Stein et al. 2005). The authors concluded that COX-associated inflammation appears to be necessary for some types of in vivo bone growth. COX inhibitors have also been shown to retard new bone formation in tooth extraction sockets (Yugoshi et al. 2002), inhibit bone fracture healing (Leonelli et al. 2006), and delay allograft healing (O'Keefe et al. 2006) in animal models. PGE_2 administration was able to counteract the osteopenic effects of cyclosporin A by inducing additional bone growth (Katz et al. 1992). Local administration of PGE has also been shown to promote bone formation in the jaw (Vrostsos et al. 2003). Human studies have indicated that high doses of NSAIDs given in conjunction with spinal fusion surgery increased the incidence of non-union (Reuben et al. 2005), and limited use of NSAIDs in patients at risk for poor fracture healing has been urged (Beck et al. 2005).

The dichotomy of PGE_2's involvement in both bone resorption and bone growth may be analogous to parathyroid hormone (PTH). PTH is a major systemic stimulus for physiologic bone resorption, necessary to maintain normal calcium levels in the blood. However, episodic doses at low concentrations are being used to enhance bone formation in osteoporotic patients, which appears to act in a manner similar to PGE_2 (Kabasawa et al. 2003). Although PGE_2 has historically been associated with bone resorption during periodontitis, the findings of the present pilot study do not support the use of PGE inhibitors as a means to enhance periodontal bone graft outcomes. These results were supported by Sculean et al. (2003), who reported that post-operative COX-2 inhibitor therapy following regenerative periodontal surgery with enamel matrix proteins did not improve clinical outcomes. Larger studies and clinical trials to evaluate how NSAIDs may impact bone-regenerative procedures should be considered in light of the dichotomous effects of PGE_2. Patients in the current study were treated post-operatively with acetaminophen/hydrocodone instead of NSAIDs to avoid this potential confounder.

In the current study, GCF MMP-9 late in wound healing had a negative correlation, although non-significant, to

6-month bone levels, suggesting that MMP-9 may not relate to bone growth in the same way as PGE_2 does. While MMP-9 has been implicated in osteogenic cell differentiation early in bone repair (Colnot et al. 2003), MMP-9 expression has been found to have a close relationship with bone graft resorption in a rabbit mandible model (Lu & Rabie 2006).

In conclusion, periodontal bone grafts using DFDBA led to an inflammatory response early in the post-operative period, resulting in elevated PGE_2 and ICTP. However, elevated PGE_2 values appeared to be associated with better bone fill outcomes, raising the possibility that post-operative use of PGE inhibitors may be counterproductive. Larger placebo-controlled randomized clinical trials would be necessary to determine the role of PGE or MMP inhibitors during post-operative care in bone-fill outcomes.

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Clinical Relevance

Scientific rationale for the study: Understanding how markers of periodontal inflammation and bone resorption behave with periodontal bone allografts may improve post-operative management, especially use of drugs that inhibit inflammation and resorption.

Principal findings: Periodontal bone allografts stimulated type 1 collagen and PGE_2 during the first 2 weeks post-operatively, and elevated prostaglandin was correlated with graft bone fill.

Practical implications: The potential of prostaglandin inhibitors to reduce bone graft success should be studied further.